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Description**Field of the invention**

[0001] The present invention generally relates to methods for reducing nitrification in soil, nitrification inhibitors and compositions comprising nitrification inhibitors. The present invention also relates to fertilisers and compositions including nitrification inhibitors for application to plants, agricultural areas (e.g. soils or pastures) to reduce or inhibit the oxidation of ammonium nitrogen to nitrite and nitrate nitrogen, such as the oxidation of ammonia- or urea-based fertilisers.

Background of the invention

[0002] High application of nitrogen fertilisers is common in agricultural systems to achieve optimal yields. However, this practice results in the release of reactive nitrogen species into the surrounding environments due to notoriously low nitrogen use efficiencies (NUEs). Plants rarely assimilate more than 50% of applied fertiliser nitrogen. In Australia, NUEs fall anywhere between 6 and 59% depending on crop type; globally NUEs have remained around 50% since the 1980's (Chen, D., et al., Australian Journal of Soil Research, 2008, 46, 289-301; Rowlings, A. W., et al., Agriculture, Ecosystems and Environment, 2016, 216, 216-225). The remaining nitrogen is vulnerable to be lost from the plant/soil system via ammonia (NH_3) volatilisation, nitrate (NO_3^-) leaching and gaseous emissions resulting from denitrification.

[0003] Of pertinent concern are losses resulting in the release of nitrous oxide (N_2O), a global warming agent 300 times more potent than carbon dioxide (CO_2), which also catalyses the destruction of stratospheric ozone. Atmospheric N_2O concentrations have increased at a rate of 0.73 ppb per year for the past three decades, and the use of nitrogen fertilisers is a leading contributor (Ciais, P., et al., Carbon and Other Biogeochemical Cycles. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA).

[0004] Ammonium (NH_4^+) in soils, either directly applied or arising indirectly from microbial conversion of nitrogen fertilisers, is quickly oxidised to nitrite (NO_2^-) and then NO_3^- through the nitrification process. NO_3^- is subsequently subjected to denitrification, where it is sequentially reduced to NO_2^- , nitric oxide (NO), N_2O and finally N_2 . Soils with high NO_3^- content are at risk of nitrogen loss via leaching of NO_3^- itself, or through gaseous losses of NO and N_2O arising from incomplete denitrification. Reducing instances of high NO_3^- concentration in soils is therefore desirable to mitigate these losses.

[0005] Slowing the conversion of NH_4^+ to NO_3^- using fertilisers amended with nitrification inhibitors is a strategy to increase NUE. Nitrification inhibitors inhibit nitrifying microbes in the soil, increasing the residence time of NH_4^+ and decreasing nitrogen losses from leaching (NO_3^-) and denitrification (N_2O , NO_x , N_2). The use of nitrification inhibitors is also recommended by the Intergovernmental Panel on Climate Change (IPCC) to mitigate N_2O emissions. Of the many compounds identified as nitrification inhibitors, the most widely researched commercial products are based on one of three chemicals: dicyandiamide (DCD, AlzChem AG), 2-chloro-6-(trichloromethyl)-pyridine (Nitrapyrin or N-Serve, Dow Chemical Co.) and 3,4-dimethylpyrazole phosphate (DMPP or ENTEC, BASF AG; the active compound is 3,4-dimethylpyrazole (DMP)). The effectiveness of these inhibitors varies greatly and appears to be influenced by environmental conditions and soil characteristics, such as pH, water content/rainfall, temperature and soil type.

[0006] DMPP is often identified as one of the more promising nitrification inhibitor candidates as it has undergone extensive toxicological testing, is effective at low concentrations and has low mobility in soils due to its positive charge (Zerulla, W., et al., Biology and Fertility of Soils, 2001, 34, 79-84). Whilst being the most promising inhibitor to date, DMPP has been found to have vastly different inhibitory activity in field studies for reducing leaching and N_2O emissions - ranging from no effect to as high as 70% inhibition for reasons not yet well understood. DMPP has shown little to no impact on improving crop/biomass yields and thus economically is not an attractive option to farmers, who ideally would offset the higher expense of the fertiliser with increased yields.

[0007] DMPP inhibitory activity is also known to be inversely related to temperature, with significant decreases in activity observed over relatively small temperature windows. Studies have shown that at a temperature of 35°C DMPP remains effective for only one week (Mahmood, T., et al., Soil Research, 2017, 55, 715-722).

[0008] It has also been reported that low pH soil conditions severely reduce DMPP activity, potentially due to the switch from the autotrophic bacteria that DMPP targets to heterotrophic bacteria predominating under these acidic conditions (Barth, G., et al., Biology and Fertility of Soils, 2001, 34, 98-102; Xi, R., et al., AMB Express, 2017, 7, 129). Attempts to circumvent some of these issues have included the reformulation of the active 3,4-dimethylpyrazole (DMP) core with succinic acid to create the isomeric mixture of 2-(N-3,4-dimethylpyrazole)succinic acid and 2-(N-4,5-dimethylpyrazole)succinic acid referred to as DMPSA. DMPSA is believed to be metabolised to the active DMP core once applied to soils, resulting in a longer lifetime in soils. WO 2010/077603 A1 discloses the use of substituted 1,2,3-

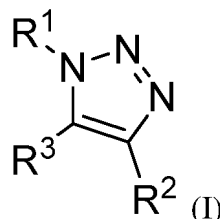
triazoles as agents for the inhibition and dispersion of biofilms in plants and BR 1020 1202 3741 A2 discloses the use of substituted 1,2,3-triazoles as pesticide.

[0009] Accordingly, there exists a need to develop new nitrification inhibitors to address the above-mentioned shortcomings.

Summary of the invention

[0010] The present invention is predicated on the discovery that substituted 1,2,3-triazoles are effective nitrification inhibitors of low volatility.

[0011] Accordingly, in one aspect the present invention provides a method for reducing nitrification in soil comprising treating the soil with a compound of Formula (I):



wherein

R¹ and R² are independently selected from optionally substituted -C₁-C₁₀alkyl, -C₂-C₁₀alkenyl, -C₂-C₁₀alkynyl, -C₁-C₁₀alkylC(O)OR⁴, -C₂-C₁₀alkenylC(O)OR⁴, -C₂-C₁₀alkynylC(O)OR⁴, -C₁-C₁₀alkylOC(O)R⁴, -C₂-C₁₀alkenylOC(O)R⁴, -C₂-C₁₀alkynylOC(O)R⁴, -C₁-C₁₀alkylOC(O)OR⁴, -C₂-C₁₀alkenylOC(O)OR⁴, -C₂-C₁₀alkynylOC(O)OR⁴, -C₁-C₁₀alkylC(O)N(R⁵R⁶), -C₂-C₁₀alkenylC(O)N(R⁵R⁶), -C₂-C₁₀alkynylC(O)N(R⁵R⁶), -C₁-C₁₀alkylNR⁵C(O)R⁶, -C₂-C₁₀alkenylNR⁵C(O)R⁶ and -C₂-C₁₀alkynylNR⁵C(O)R⁶;

R³ is H or is selected from optionally substituted -C₁-C₁₀alkyl, -C₂-C₁₀alkenyl, -C₂-C₁₀alkynyl, -C₁-C₁₀alkylC(O)OR⁴, -C₂-C₁₀alkenylC(O)OR⁴, -C₂-C₁₀alkynylC(O)OR⁴, -C₁-C₁₀alkylOC(O)R⁴, -C₂-C₁₀alkenylOC(O)R⁴, -C₂-C₁₀alkynylOC(O)R⁴, -C₁-C₁₀alkylOC(O)OR⁴, -C₂-C₁₀alkenylOC(O)OR⁴, -C₂-C₁₀alkynylOC(O)OR⁴, -C₁-C₁₀alkylC(O)N(R⁵R⁶), -C₂-C₁₀alkenylC(O)N(R⁵R⁶), -C₂-C₁₀alkynylC(O)N(R⁵R⁶), -C₁-C₁₀alkylNR⁵C(O)R⁶, -C₂-C₁₀alkenylNR⁵C(O)R⁶ and -C₂-C₁₀alkynylNR⁵C(O)R⁶;

R⁴ is selected from -C₁-C₄alkyl, -C₂-C₄alkenyl and -C₂-C₄alkynyl; and

R⁵ and R⁶ are independently selected from H, -C₁-C₄alkyl, -C₂-C₄alkenyl and -C₂-C₄alkynyl;

or agriculturally acceptable salts thereof.

[0012] Preferably, the method includes the step of co-treating the soil with a urease inhibitor.

[0013] Preferably, the soil is co-treated with a fertiliser.

[0014] In another aspect, the present invention provides the use of a composition for reducing nitrification comprising a compound of Formula (I) as defined above and at least one agriculturally acceptable adjuvant or diluent, the composition optionally including a urease inhibitor.

[0015] In a further aspect, the present invention provides a fertiliser comprising a urea- or ammonium-based fertiliser and a compound of Formula (I) as defined herein.

[0016] In a yet further aspect, the present invention provides the use of a compound for reducing nitrification comprising a compound of Formula (I) as defined in Claim 1 or Claim 2.

[0017] Optionally, R¹ and/or R² are substituted with one or more amino, hydroxy, C₁-C₄alkoxy- or a 3-10-membered monocyclic or fused bicyclic heteroaryl comprising one or more heteroatoms selected from N, O and S, wherein said heteroaryl is optionally substituted with one or more C₁-C₁₀alkyl, oxo, hydroxy, C₁-C₄alkoxy- or amino and/or R³ is substituted with one or more amino, hydroxy, C₁-C₄alkoxy- or a 3-10-membered monocyclic or fused bicyclic heteroaryl comprising one or more heteroatoms selected from N, O and S, wherein said heteroaryl is optionally substituted with one or more C₁-C₁₀alkyl, oxo, hydroxy, C₁-C₄alkoxy- or amino.

[0018] In a still yet further embodiment, there is provided a fertiliser as defined above wherein the urea- or ammonium-based fertiliser is in the form of a granule and the compound of Formula (I) and a urease inhibitor are coated on the granule.

[0019] These and other aspects of the present invention will become more apparent to the skilled addressee upon reading the following detailed description in connection with the accompanying examples and claims.

Brief Description of the Drawings

[0020] The invention will herein be described by way of example only with reference to the following non-limiting Figures in which:

Figure 1 illustrates the measured $\text{NH}_4^+\text{-N}$ (A, C) and $\text{NO}_x^-\text{-N}$ (B, D) concentrations of Horsham soil incubated at 25°C (A, B) and 35°C (C, D) following treatment with: $(\text{NH}_4)_2\text{SO}_4$ [•], $(\text{NH}_4)_2\text{SO}_4$ + H-DMPP [■], $(\text{NH}_4)_2\text{SO}_4$ + 13 [♦], $(\text{NH}_4)_2\text{SO}_4$ + 14 [◊], $(\text{NH}_4)_2\text{SO}_4$ + 16 [□]. Inhibition of nitrification is indicated by a slow decrease of $\text{NH}_4^+\text{-N}$ and slow increase of $\text{NO}_x^-\text{-N}$.

Figure 2 illustrates calculated $\text{NO}_x^-\text{-N}$ production rates (mg $\text{NO}_x^-\text{-N}$ /kg soil/day) after 28-day incubation in the Horsham soil (pH 8.8) at 25°C and 35°C. All samples were treated with the fertiliser $(\text{NH}_4)_2\text{SO}_4$ at a rate of 100 mg N kg⁻¹ on day 0. Values presented are means (n = 3); errors are standard errors of the mean. Inhibition of nitrification is indicated by slow $\text{NO}_x^-\text{-N}$ production rates.

Figure 3 illustrates the measured $\text{NH}_4^+\text{-N}$ (A, C) and $\text{NO}_x^-\text{-N}$ (B, D) concentrations of Dahlen soil incubated at 25°C (A, B) and 35°C (C, D) following treatment with: $(\text{NH}_4)_2\text{SO}_4$ [•], $(\text{NH}_4)_2\text{SO}_4$ + H-DMPP [■], $(\text{NH}_4)_2\text{SO}_4$ + 13 [♦], $(\text{NH}_4)_2\text{SO}_4$ + 16 [□]. Inhibition of nitrification is indicated by a slow decrease of $\text{NH}_4^+\text{-N}$ and slow increase of $\text{NO}_x^-\text{-N}$.

Figure 4 illustrates the calculated $\text{NO}_x^-\text{-N}$ production rates (mg $\text{NO}_x^-\text{-N}$ /kg soil/day) after 28-day incubations in the Dahlen soil (pH 7.3) at 25°C and 35°C. All samples were treated with the fertiliser $(\text{NH}_4)_2\text{SO}_4$ at a rate of 100 mg N kg⁻¹ on day 0. Values presented are means (n = 3); errors are standard errors of the mean. Inhibition of nitrification is indicated by slow $\text{NO}_x^-\text{-N}$ production rates.

Figure 5 illustrates the measured $\text{NH}_4^+\text{-N}$ (A, C) and $\text{NO}_x^-\text{-N}$ (B, D) concentrations of Dahlen soil incubated at 25°C (A, B) and 35°C (C, D) following treatment with: $(\text{NH}_4)_2\text{SO}_4$ [●], $(\text{NH}_4)_2\text{SO}_4$ + H-DMPP [■], $(\text{NH}_4)_2\text{SO}_4$ + 18 [◊], $(\text{NH}_4)_2\text{SO}_4$ + 20 [▽], $(\text{NH}_4)_2\text{SO}_4$ + 23 [◊]. Inhibition of nitrification is indicated by a slow decrease of $\text{NH}_4^+\text{-N}$ and slow increase of $\text{NO}_x^-\text{-N}$.

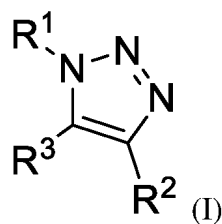
Figure 6 illustrates the measured $\text{NH}_4^+\text{-N}$ (A, C) and $\text{NO}_x^-\text{-N}$ (B, D) concentrations of South Johnstone soil incubated at 25°C (A, B) and 35°C (C, D) following treatment with: $(\text{NH}_4)_2\text{SO}_4$ [•], $(\text{NH}_4)_2\text{SO}_4$ + H-DMPP [■], $(\text{NH}_4)_2\text{SO}_4$ + 3 [♦], $(\text{NH}_4)_2\text{SO}_4$ + 16 [□], $(\text{NH}_4)_2\text{SO}_4$ + 18 [◊]. Inhibition of nitrification is indicated by a slow decrease of $\text{NH}_4^+\text{-N}$ and slow increase of $\text{NO}_x^-\text{-N}$.

Figure 7 illustrates the results of soil TLC leaching of inhibitor compounds DMP and Compound 16 in Dahlen soil (A) or South Johnstone soil (B). Higher R_f values indicate higher degrees of leachability through the soil profile.

Detailed description of the invention

[0021] Mono-, di- and trisubstituted 1,2,3-triazoles were investigated as potential nitrification inhibitors. Substituted 1,2,3-triazoles were seen as a good candidate as they are synthetically readily accessible using copper-catalysed click chemistry approaches and have found application in medicinal and pharmacological fields as a pharmacophore, due to their broad biological activities. Variation of the substitution pattern at the 1, 4 and/or 5 positions allows for optimisation of any inhibitory activity. It is believed that varying the substituents and substitution pattern may enable tailoring of the nitrification inhibitors for certain soils such as acid, neutral and alkaline soils as well as for different climatic conditions.

[0022] In one embodiment, the invention provides a method for reducing nitrification in soil comprising treating the soil with a compound of Formula (I):



wherein

R^1 and R^2 are independently selected from optionally substituted $-C_1-C_{10}$ alkyl, $-C_2-C_{10}$ alkenyl, $-C_2-C_{10}$ alkynyl, $-C_1-C_{10}$ alkylC(O)OR⁴, $-C_2-C_{10}$ alkenylC(O)OR⁴, $-C_2-C_{10}$ alkynylC(O)OR⁴, $-C_1-C_{10}$ alkylOC(O)R⁴, $-C_2-C_{10}$ alkenylOC(O)R⁴, $-C_2-C_{10}$ alkynylOC(O)R⁴, $-C_1-C_{10}$ alkylC(O)N(R⁵R⁶), $-C_2-C_{10}$ alkenylC(O)N(R⁵R⁶), $-C_2-C_{10}$ alkynylC(O)N(R⁵R⁶), $-C_1-C_{10}$ alkylNR⁵C(O)R⁶, $-C_2-C_{10}$ alkenylNR⁵C(O)R⁶ and $-C_2-C_{10}$ alkynylNR⁵C(O)R⁶;

R^3 is H or is selected from optionally substituted $-C_1-C_{10}$ alkyl, $-C_2-C_{10}$ alkenyl, $-C_2-C_{10}$ alkynyl, $-C_1-C_{10}$ alkylC(O)OR⁴, $-C_2-C_{10}$ alkenylC(O)OR⁴, $-C_2-C_{10}$ alkynylC(O)OR⁴, $-C_1-C_{10}$ alkylOC(O)R⁴, $-C_2-C_{10}$ alkenylOC(O)R⁴, $-C_2-C_{10}$ alkynylOC(O)R⁴, $-C_1-C_{10}$ alkylC(O)N(R⁵R⁶), $-C_2-C_{10}$ alkenylC(O)N(R⁵R⁶), $-C_2-C_{10}$ alkynylC(O)N(R⁵R⁶), $-C_1-C_{10}$ alkylNR⁵C(O)R⁶, $-C_2-C_{10}$ alkenylNR⁵C(O)R⁶ and $-C_2-C_{10}$ alkynylNR⁵C(O)R⁶;

R^4 is selected from $-C_1-C_4$ alkyl, $-C_2-C_4$ alkenyl and $-C_2-C_4$ alkynyl; and

R^5 and R^6 are independently selected from H, $-C_1-C_4$ alkyl, $-C_2-C_4$ alkenyl and $-C_2-C_4$ alkynyl;

or agriculturally acceptable salts thereof.

[0023] In one embodiment, with reference to Formula (I), R^1 and R^2 are independently selected from $-C_1-C_{10}$ alkyl, $-C_2-C_{10}$ alkenyl, $-C_2-C_{10}$ alkynyl, $-C_1-C_{10}$ alkylC(O)OR⁴, $-C_2-C_{10}$ alkenylC(O)OR⁴, $-C_2-C_{10}$ alkynylC(O)OR⁴, $-C_1-C_{10}$ alkylOC(O)R⁴, $-C_2-C_{10}$ alkenylOC(O)R⁴, $-C_2-C_{10}$ alkynylOC(O)R⁴, $-C_1-C_{10}$ alkylC(O)N(R⁵R⁶), $-C_2-C_{10}$ alkenylC(O)N(R⁵R⁶), $-C_2-C_{10}$ alkynylC(O)N(R⁵R⁶), $-C_1-C_{10}$ alkylNR⁵C(O)R⁶, $-C_2-C_{10}$ alkenylNR⁵C(O)R⁶ and $-C_2-C_{10}$ alkynylNR⁵C(O)R⁶ optionally substituted with one or more amino, hydroxy, C_1-C_4 alkoxy- or a 3-10-membered monocyclic or fused bicyclic heteroaryl comprising one or more heteroatoms selected from N, O and S, wherein said heteroaryl is optionally substituted with one or more C_1-C_{10} alkyl, oxo, hydroxy, C_1-C_4 alkoxy- or amino;

R^3 is H or is selected from $-C_1-C_{10}$ alkyl, $-C_2-C_{10}$ alkenyl, $-C_2-C_{10}$ alkynyl, $-C_1-C_{10}$ alkylC(O)OR⁴, $-C_2-C_{10}$ alkenylC(O)OR⁴, $-C_2-C_{10}$ alkynylC(O)OR⁴, $-C_1-C_{10}$ alkylOC(O)R⁴, $-C_2-C_{10}$ alkenylOC(O)R⁴, $-C_2-C_{10}$ alkynylOC(O)R⁴, $-C_1-C_{10}$ alkylC(O)N(R⁵R⁶), $-C_2-C_{10}$ alkenylC(O)N(R⁵R⁶), $-C_2-C_{10}$ alkynylC(O)N(R⁵R⁶), $-C_1-C_{10}$ alkylNR⁵C(O)R⁶, $-C_2-C_{10}$ alkenylNR⁵C(O)R⁶ and $-C_2-C_{10}$ alkynylNR⁵C(O)R⁶ optionally substituted with one or more amino, hydroxy, C_1-C_4 alkoxy- or a 3-10-membered monocyclic or fused bicyclic heteroaryl comprising one or more heteroatoms selected from N, O and S, wherein said heteroaryl is optionally substituted with one or more C_1-C_{10} alkyl, oxo, hydroxy, C_1-C_4 alkoxy- or amino;

R^4 is selected from $-C_1-C_4$ alkyl, $-C_2-C_4$ alkenyl and $-C_2-C_4$ alkynyl; and

R^5 and R^6 are independently selected from H, $-C_1-C_4$ alkyl, $-C_2-C_4$ alkenyl and $-C_2-C_4$ alkynyl.

[0024] In this specification, unless otherwise defined, the term "optionally substituted" is taken to mean that a group may or may not be further substituted with one or more groups selected from hydroxyl, alkyl, alkoxy, alkoxycarbonyl, alkoxycarbonyloxy, alkenyl, alkenyloxy, alkynyl, alkynyloxy, amino, aminoacyl, amido, thio, arylalkyl, arylalkoxy, aryl, aryloxy, acylamino, carboxy, cyano, halogen, nitro, sulfo, phosphono, phosphorylamino, phosphinyl, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, trihalomethyl, pentafluoroethyl, trifluoromethoxy, difluoromethoxy, trifluoromethanethio, trifluoroethenyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamine, mono- and di-heteroarylamine, mono- and di-heterocyclylamine, unsymmetric di-substituted amines having different substituents selected from alkyl, aryl, heteroaryl and heterocyclyl, mono- and di-alkylamido, mono- and di-(substi-

tuted alkyl)amido, mono- and di-arylamido, mono- and di-heteroarylamido, mono- and di-heterocyclamido, unsymmetric di-substituted amides having different substituents selected from alkyl, aryl, heteroaryl and heterocycl.

[0025] As used herein, the term "alkyl", used either alone or in compound words, denotes straight chain or branched alkyl. Prefixes such as "C₂-C₁₀" are used to denote the number of carbon atoms within the alkyl group (from 2 to 10 in this case). Examples of straight chain and branched alkyl include methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *sec*-butyl, *t*-butyl, *n*-pentyl, hexyl, heptyl, 5-methylheptyl, 5-methylhexyl, octyl, nonyl, decyl, undecyl, dodecyl and docosyl (C₂₂).

[0026] As used herein, the term "alkenyl", used either alone or in compound words, denotes straight chain or branched hydrocarbon residues containing at least one carbon to carbon double bond including ethylenically mono-, di- or polyunsaturated alkyl groups as previously defined. Prefixes such as "C₂-C₂₀" are used to denote the number of carbon atoms within the alkenyl group (from 2 to 20 in this case). Examples of alkenyl include vinyl, allyl, 1-methylvinyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, 1-hexenyl, 3-hexenyl, 1-heptenyl, 3-heptenyl, 1-octenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 3-decenyl, 1,3-butadienyl, 1,4-pentadienyl, 1,3-hexadienyl, 1,4-hexadienyl and 5-docosenyl (C₂₂).

[0027] As used herein, the term "alkynyl", used either alone or in compound words, denotes straight chain or branched hydrocarbon residues containing at least one carbon to carbon triple bond. Prefixes such as "C₂-C₂₀" are used to denote the number of carbon atoms within the alkynyl group (from 2 to 20 in this case).

[0028] The term "amino" as used herein refers to a nitrogen atom substituted with, for example, hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl or combinations thereof.

[0029] The term "amido" as used herein refers to an amide group, i.e. a group of the formula - C(O)NH₂. The group is bonded to the remainder of the molecule via the carbonyl carbon atom. The nitrogen atom may also be substituted with, for example, alkyl, alkenyl, alkynyl, aryl, heteroaryl or combinations thereof.

[0030] The term "aryl" refers to aromatic monocyclic (e.g. phenyl) or polycyclic groups (e.g. tricyclic, bicyclic, e.g., naphthalene, anthryl, phenanthryl). Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (e.g. tetralin, methylenedioxyphenyl).

[0031] The term "heteroaryl", as used herein, represents a monocyclic or bicyclic ring, typically of up to 7 atoms in each ring, wherein at least one ring is aromatic and contains from 1 to 4 heteroatoms selected from the group consisting of O, N and S. Heteroaryl groups within the scope of this definition include but are not limited to: benzimidazole (otherwise known as benzoimidazole), acridinyl, carbazoyl, cinnolinyl, quinoxalinyl, pyrazolyl, indolyl, benzotriazolyl, furanyl, thienyl, benzothienyl, benzofuranyl, quinolinyl, isoquinolinyl, oxazolyl, isoxazolyl, indoiyl, pyrazinyl, pyridazinyl, pyridinyl, pyrimidinyl, pyrrolyl, tetrahydroquinoline. As with the definition of heterocycle below, "heteroaryl" is also understood to include the *N*-oxide derivative of any nitrogen-containing heteroaryl. In cases where the heteroaryl substituent is bicyclic and one ring is non-aromatic or contains no heteroatoms, it is understood that attachment is via the aromatic ring or via the heteroatom containing ring, respectively.

[0032] The term "heteroatom" includes atoms of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

[0033] The term "alkoxy" includes substituted and unsubstituted alkyl, alkenyl, and alkynyl groups covalently linked to an oxygen atom. Examples of alkoxy groups include methoxy, ethoxy, isopropoxy (isopropoxy), propoxy, butoxy, and pentoxy groups and may include cyclic groups such as cyclopentoxy.

[0034] In one embodiment, the method as defined above comprises co-treating the soil with a fertiliser.

[0035] In another embodiment, the method as defined above is effective for reducing nitrification in soil in an elevated ambient temperature, for example, an ambient temperature of between about 25°C and about 50°C, such as between about 30°C and about 45°C.

[0036] It will be appreciated that a fertiliser may be formulated to contain a mixture of minerals and nutrients where a source of nitrogen simply provides one of the many minerals and nutrients present in the fertiliser. The fertiliser may be a nitrogen-based fertiliser. The nitrogen-based fertiliser may be an ammonium, ammonium nitrate or urea-based fertiliser, or comprise ammonia, ammonium, nitrate or urea (or may contain all three forms as is the case with urea ammonium nitrate). The nitrogen-based fertiliser may be an organic or inorganic fertiliser. The organic fertiliser may include animal waste. In one embodiment, the fertiliser comprises or consists of an ammonium-based fertiliser. In another embodiment, the fertiliser comprises or consists of a urea-based fertiliser.

[0037] In one embodiment, the fertilisers are inorganic fertilisers. These can be ammonium- or urea-containing fertilisers. Examples of ammonium-containing fertilisers of this type are NPK fertilisers, calcium ammonium nitrate, ammonium sulfate nitrate, ammonium sulfate or ammonium phosphate. In a particular embodiment, the ammonium-containing fertilisers are selected from the group consisting of anhydrous ammonia, ammonium sulfate, urea, ammonium nitrate, ammonium phosphate and mixtures thereof.

[0038] The fertiliser may be coated or impregnated with the nitrification inhibitor or formulation thereof. The fertiliser may be in the form of granules, crystals or powder incorporating the nitrification inhibitor or formulation thereof. The fertiliser may be a liquid fertiliser comprising the nitrification inhibitor or formulation thereof. It will be appreciated that other forms of fertiliser may be used.

[0039] Accordingly, in one embodiment the present invention provides a fertiliser as defined above wherein the urea- or ammonium-based fertiliser is in the form of a granule and the compound of Formula (I) is coated on the granule.

[0040] In a further embodiment, the method as defined above comprises co-treating the soil with a urease inhibitor.

[0041] Currently, there is only one commercially available urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT, marketed as Agrotain). Unfortunately, the lifetime of this inhibitor in soils is limited. The major degradation pathway in acidic and slightly alkaline soils is chemical hydrolysis, whereas microbial degradation becomes dominant in more alkaline soils.

[0042] Most soil conditions would benefit from fertilisers that contain both urease and nitrification inhibitors. Furthermore, recent research suggests that, while nitrification inhibitors are effective in reducing N_2O emission from various agricultural systems, they may increase NH_3 emission under certain conditions. These problems highlight the importance of using both urease and nitrification inhibitors in mitigating nitrogen loss. At present, there are limited commercial products which combine both urease and nitrification inhibitors, since production is hampered by the challenge of combining acid-sensitive NBPT with the acidic DMPP.

[0043] In one embodiment, there is provided a fertiliser as defined above wherein the urea- or ammonium-based fertiliser is in the form of a granule and the compound of Formula (I) and a urease inhibitor are coated on the granule.

[0044] It will be understood that the compounds used in the method of the invention may exist in a plurality of equivalent tautomeric forms. For the sake of clarity, the compounds have been depicted as single tautomers, despite all such tautomeric forms being considered within the scope of the invention.

[0045] The structures of some of the compounds used in the method of the invention may include asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry (e.g., all enantiomers, stereoisomers, rotamers, tautomers, diastereomers, or racemates) are included within the scope of this invention. The present invention includes within its scope all of these stereoisomeric forms either isolated (in, for example, enantiomeric isolation), or in combination (including racemic mixtures and diastereomeric mixtures).

[0046] The skilled person will appreciate that there are a range of techniques available to produce achiral compounds used in the method of the invention in racemic, enantioenriched or enantiopure forms. For example, enantioenriched or enantiopure forms of the compounds may be produced through stereoselective synthesis and/or through the use of chromatographic or selective recrystallisation techniques.

[0047] The compounds used in the method of the invention may be in crystalline form, may be oils or may be solvates (e.g. hydrates), and it is intended that all forms are within the scope of the present invention. The term "solvate" is a complex of variable stoichiometry formed by a solute (in this invention, a compound used in the method of the invention) and a solvent. Such solvents should preferably not interfere with the biological activity of the solute. Solvents may be, by way of example, water, acetone, ethanol or acetic acid. Methods of solvation are generally known within the art.

[0048] The compounds used in the method of the invention that have at least one basic centre can form acid addition salts. Acid addition salts may be prepared from inorganic and organic acids. Examples of inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like. Examples of organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0049] The compounds which have at least one acidic group can form base addition salts. Base addition salts may be prepared from inorganic and organic bases. Corresponding counterions derived from inorganic bases include the sodium, potassium, lithium, ammonium, calcium and magnesium salts. Organic bases include primary, secondary and tertiary amines, substituted amines including naturally-occurring substituted amines, and cyclic amines, including isopropylamine, trimethyl amine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, and N-ethylpiperidine.

[0050] In a further aspect there is provided a composition for reducing nitrification in soil comprising a compound of Formula (I) as defined herein and at least one agriculturally acceptable adjuvant or diluent.

[0051] The compounds used in the method according to the invention can be used as nitrification inhibitors in unmodified form but are generally formulated into compositions in various ways using formulation adjuvants, such as carriers, solvents and surface-active substances. The formulations can be in various physical forms, for example, in the form of dusting powders, gels, wettable powders, water-dispersible granules, water-dispersible tablets, effervescent pellets, emulsifiable concentrates, microemulsifiable concentrates, oil-in-water emulsions, oil-flowables, aqueous dispersions, oily dispersions, suspo-emulsions, capsule suspensions, emulsifiable granules, soluble liquids, water-soluble concentrates (with water or a water-miscible organic solvent as carrier), impregnated polymer films or in other known forms. Such formulations can either be used directly or diluted prior to use. The dilutions can be made, for example, with a diluent selected from but not limited to water, liquid fertilisers, micronutrients, biological organisms, oil or solvents.

[0052] The formulations can be prepared by mixing the nitrification inhibitor used in the method of the invention with the formulation adjuvants in order to obtain compositions in the form of finely divided solids, granules, solutions, dispersions or emulsions. The nitrification inhibitors can also be formulated with other adjuvants, such as finely divided solids, mineral oils, oils of vegetable or animal origin, modified oils of vegetable or animal origin, organic solvents, water, surface-active substances or combinations thereof.

[0053] The nitrification inhibitors can also be contained in very fine microcapsules. Microcapsules contain the active ingredients in a porous carrier to enable release of the nitrification inhibitors into the environment in controlled amounts (e.g. slow-release). Microcapsules usually have a diameter of from 0.1 to 500 microns. They contain active ingredients in an amount of about from 25 to 95% by weight of the capsule weight. The active ingredients can be in the form of a monolithic solid, in the form of fine particles in solid or liquid dispersion or in the form of a suitable solution. The encapsulating membranes can comprise, for example, natural or synthetic rubbers, cellulose, styrene/butadiene copolymers, polyacrylonitriles, polyacrylates, polyesters, polyamides, polyureas, polyurethanes or chemically modified polymers and starch xanthates or other polymers that are known to the person skilled in the art. Alternatively, very fine microcapsules can be formed in which the active ingredient is contained in the form of finely divided particles in a solid matrix of base substance, but the microcapsules are not themselves encapsulated.

[0054] Formulation adjuvants that are suitable for the preparation of the compositions used in the method according to the invention are known in the art. As liquid carriers there may be used: water, toluene, xylene, petroleum ether, vegetable oils, acetone, methyl ethyl ketone, sulfolane (tetramethylene sulfone), cyclohexanone, acid anhydrides, acetonitrile, acetophenone, amyl acetate, 2-butanone, butylene carbonate, chlorobenzene, cyclohexane, cyclohexanol, alkyl esters of acetic acid, diacetone alcohol, 1,2-dichloropropane, diethanolamine, p-diethylbenzene, diethylene glycol, diethylene glycol abietate, diethylene glycol butyl ether, diethylene glycol ethyl ether, diethylene glycol methyl ether, N,N-dimethylformamide, dimethyl sulfoxide, 1,4-dioxane, dipropylene glycol, dipropylene glycol methyl ether, dipropylene glycol dibenzoate, diproxitol, alkylpyrrolidone, ethyl acetate, 2-ethylhexanol, ethylene carbonate, 1,1,1-trichloroethane, 2-heptanone, alpha-pinene, d-limonene, ethyl lactate, ethylene glycol, ethylene glycol butyl ether, ethylene glycol methyl ether, gamma-butyrolactone, glycerol, glycerol acetate, glycerol diacetate, glycerol triacetate, hexadecane, hexylene glycol, isoamyl acetate, isobornyl acetate, isooctane, isophorone, isopropylbenzene, isopropyl myristate, lactic acid, laurylamine, mesityl oxide, methoxypropanol, methyl isoamyl ketone, methyl isobutyl ketone, methyl laurate, methyl octanoate, methyl oleate, methylene chloride, m-xylene, n-hexane, n-octylamine, octadecanoic acid, octylamine acetate, oleic acid, oleylamine, o-xylene, phenol, polyethylene glycol, propionic acid, propyl lactate, propylene carbonate, propylene glycol, propylene glycol methyl ether, p-xylene, toluene, triethyl phosphate, triethylene glycol, xylenesulfonic acid, paraffin, mineral oil, trichloroethylene, perchloroethylene, ethyl acetate, amyl acetate, butyl acetate, propylene glycol methyl ether, diethylene glycol methyl ether, methanol, ethanol, isopropanol, and alcohols of higher molecular weight, such as amyl alcohol, tetrahydrofurfuryl alcohol, hexanol, octanol, ethylene glycol, propylene glycol, glycerol, N-methyl-2-pyrrolidone and the like.

[0055] Suitable solid carriers are, for example, talc, titanium dioxide, pyrophyllite clay, silica, attapulgite clay, kieselguhr, limestone, calcium carbonate, bentonite, calcium montmorillonite, cottonseed husks, wheat flour, soybean flour, pumice, wood flour, ground walnut shells, lignin and similar substances.

[0056] A large number of surface-active substances can advantageously be used in both solid and liquid formulations, especially in those formulations which can be diluted with a carrier prior to use. Surface-active substances may be anionic, cationic, non-ionic or polymeric, and they can be used as emulsifiers, wetting agents or suspending agents or for other purposes. Typical surface-active substances include, for example, salts of alkyl sulfates, such as diethanolammonium lauryl sulfate; salts of alkylarylsulfonates, such as calcium dodecylbenzenesulfonate; alkylphenol/alkylene oxide addition products, such as nonylphenol ethoxylate; alcohol/alkylene oxide addition products, such as tridecylalcohol ethoxylate; soaps, such as sodium stearate; salts of alkyl naphthalenesulfonates, such as sodium dibutyl naphthalenesulfonate; dialkyl esters of sulfosuccinate salts, such as sodium di(2-ethylhexyl)sulfosuccinate; sorbitol esters, such as sorbitol oleate; quaternary amines, such as lauryltrimethylammonium chloride, polyethylene glycol esters of fatty acids, such as polyethylene glycol stearate; block copolymers of ethylene oxide and propylene oxide; and salts of mono- and di-alkylphosphate esters; and also further substances described e.g. in McCutcheon's Detergents and Emulsifiers Annual, MC Publishing Corp., Ridgewood N.J. (1981).

[0057] Further adjuvants that can be used in nitrification inhibitor formulations include crystallisation inhibitors, viscosity modifiers, suspending agents, dyes, anti-oxidants, foaming agents, light absorbers, mixing auxiliaries, anti-foams, complexing agents, neutralising or pH-modifying substances and buffers, corrosion inhibitors, fragrances, wetting agents, take-up enhancers, micronutrients, plasticisers, glidants, lubricants, dispersants, thickeners, anti-freezes, microbicides, and liquid and solid fertilisers.

[0058] The compositions used in the method according to the invention can include an additive comprising an oil of vegetable or animal origin, a mineral oil, alkyl esters of such oils or mixtures of such oils and oil derivatives. The amount of oil additive in the composition used in the method according to the invention is generally from 0.01 to 10%, based on the mixture to be applied. As an example, the oil additive can be added to a spray tank in the desired

concentration after a spray mixture has been prepared. Preferred oil additives comprise mineral oils or an oil of vegetable origin, for example rapeseed oil, olive oil or sunflower oil, emulsified vegetable oil, alkyl esters of oils of vegetable origin, for example, the methyl derivatives, or an oil of animal origin, such as fish oil or beef tallow. Preferred oil additives comprise alkyl esters of C₈-C₂₂ fatty acids, especially the methyl derivatives of C₁₂-C₁₈ fatty acids, for example the methyl esters of lauric acid, palmitic acid and oleic acid (methyl laurate, methyl palmitate and methyl oleate, respectively).

[0059] The compositions used in the method according to the invention generally comprise from 0.1 to 99% by weight, especially from 0.1 to 95% by weight, of compounds of the present invention and from 1 to 99.9% by weight of a formulation adjuvant which may include from 0 to 25% by weight of a surface-active substance. Whereas commercial products may preferably be formulated as concentrates, the end user will normally employ dilute formulations.

[0060] The rates of application vary within wide limits and depend on the nature of the soil, the method of application, the crop plant, the type of fertiliser used, the prevailing climatic conditions, and other factors governed by the method of application, the time of application and the target crop. As a general guideline compounds may be applied at a rate of from 1 to 2000 L/ha, especially from 10 to 1000 L/ha.

[0061] The composition may further comprise a urease inhibitor.

[0062] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0063] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[0064] The invention will now be described with reference to the following non-limiting examples:

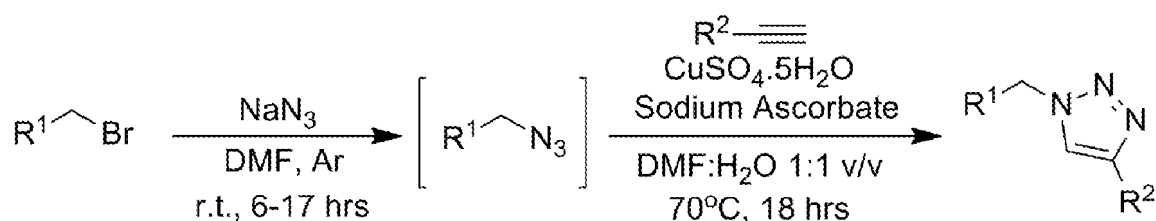
1. Synthesis of Nitrification Inhibitors

1.1 General.

[0065] Reaction progress was monitored by thin-layer chromatography (TLC) using silica gel 60 aluminium-backed plates coated with fluorescent indicator F254 (Merck). Plates were visualised using UV irradiation (254 nm) alone or in conjunction with ninhydrin-, potassium permanganate- or iodine-based stains. Purification by silica gel chromatography was performed using Davisil Chromatographic Silica Media LC60A 40-63 micron, with solvent systems as specified. All ¹H and ¹³C NMR spectra were recorded on a 400 MHz Varian INOVA spectrometer (at 400 or 101 MHz, respectively) downfield from residual solvents peaks using solvent resonances as the internal standard (¹H NMR: CDCl₃ at 7.26 ppm, DMSO-d₆ at 2.50 ppm; ¹³C NMR: CDCl₃ at 77.0 ppm, DMSO-d₆ at 39.5 ppm). Chemical shifts are reported in parts per million (ppm, δ), with the splitting patterns indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; dd, doublet of doublets. The coupling constants, *J*, are reported in Hertz (Hz). Electrospray ionization high resolution mass spectrometry (HRMS) was performed on a Thermo Scientific Exactive Plus Orbitrap mass spectrometer (Thermo, Bremen, German) operated in positive mode.

1.2 General Procedure A: Copper(I)-catalysed azide-alkyne cycloaddition (CuAAC) to synthesise 1,4-disubstituted triazoles

[0066]

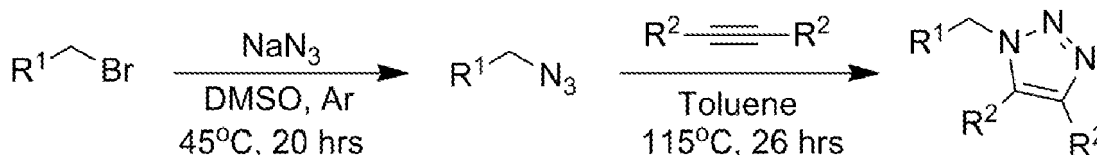


Scheme 1: Reaction scheme for General Procedure A.

[0067] Sodium azide (1.2 or 1.5 equiv.) was suspended in DMF (0.85 M) in a flask under argon atmosphere, and to this the appropriate alkyl bromide (1 eq.) was added. The solution was stirred at room temperature for 6-17 hours. The reaction was quenched by the addition of H₂O (DMF/H₂O, 1:1 v/v), before the successive additions of CuSO₄·5H₂O (0.06 equiv.), sodium ascorbate (0.3 equiv.) and the appropriate alkyne (1.2 or 1.5 equiv.). The reaction was heated at 70°C overnight with vigorous stirring. The reaction was cooled to room temperature before dilution with H₂O (at least 3 x DMF volume) and extraction with ethyl acetate. The extracts were combined, washed with 5% aq. LiCl solution and concentrated before purification by silica chromatography.

1.3 General Procedure B: Thermal Huisgen 1,3-dipolar cycloaddition to synthesise 1,4,5-trisubstituted triazoles

[0068]



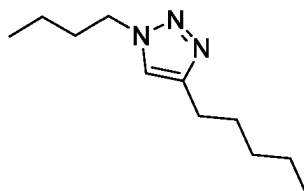
Scheme 2: Reaction scheme for General Procedure B.

[0069] Sodium azide (1.5 equiv.) and appropriate alkyl bromide (1 equiv.) were charged into a flask flushed with argon. They were suspended in DMSO (1.28 M) and warmed to 45°C with vigorous stirring. The reaction was cooled to room temperature after 20 hours and quenched with H₂O (DMF/H₂O, 4:5 v/v), before extraction with ether. The ethereal extracts were concentrated under N₂ flow to an oil, which was used directly in the subsequent step. *CAUTION: Organic azides may be explosive, do not evaporate to dryness. Smaller azides were handled using solvent substitution, where toluene was added before ether was evaporated under N₂ flow.

[0070] The crude azide was suspended in toluene (0.21 M) before addition of the appropriate internal alkyne (1.1 equiv.). The reaction was then heated at 115°C with vigorous stirring. Once completed by TLC (24 to 48 hrs), the reaction was cooled. Toluene was removed *in vacuo* to leave crude triazole as a waxy brown solid. Purification of the crude product was achieved through recrystallisation or column chromatography.

1.4 Synthesis of 1-butyl-4-pentyl-1H-1,2,3-triazole (1)

[0071]



[0072] Synthesised from General Procedure A; **1** (2.14 g, 11.0 mmol, 64%) was obtained starting from sodium azide (25.6 mmol), 1-bromobutane (17.1 mmol), CuSO₄·5H₂O (1.0 mmol), sodium ascorbate (5.1 mmol) and 1-heptyne (25.5 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 4:1; R_f = 0.27).

[0073] Yield: 64% (colourless liquid).

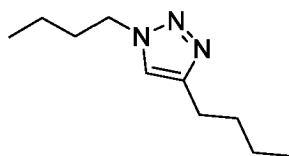
[0074] ¹H NMR (400 MHz, CDCl₃): δ 7.22 (s, 1H), 4.26 (t, J = 7.3 Hz, 2H), 2.69 - 2.60 (m, 2H), 1.82 (p, J = 7.4 Hz, 2H), 1.61 (p, J = 7.4 Hz, 2H), 1.37 - 1.21 (m, 6H), 0.89 (t, J = 7.4 Hz, 3H), 0.88 - 0.80 (m, 3H).

[0075] ¹³C NMR (101 MHz, CDCl₃): δ 148.30, 120.32, 49.78, 32.27, 31.40, 29.14, 25.61, 22.35, 19.66, 13.93, 13.40.

[0076] HRMS (ESI +) m/z: [C₁₁H₂₁N₃ + H]⁺ calculated 196.18082, found 196.18098.

1.5 Synthesis of 1,4-butyl-1H-1,2,3-triazole (2)

[0077]



[0078] Synthesised from General Procedure A; **2** (2.33 g, 12.9 mmol, 75%) was obtained starting from sodium azide (25.6 mmol), 1-bromobutane (17.1 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.0 mmol), sodium ascorbate (5.1 mmol) and 1-hexyne (25.5 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 4:1; R_f = 0.19).

[0079] **Yield:** 75% (colourless liquid).

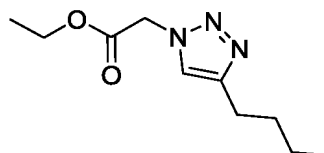
[0080] **^1H NMR** (400 MHz, CDCl_3): δ 7.22 (s, 1H), 4.25 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 7.8 Hz, 2H), 1.81 (p, J = 7.4 Hz, 2H), 1.59 (p, J = 7.6 Hz, 2H), 1.38 - 1.23 (m, 4H), 0.93 - 0.83 (m, 6H).

[0081] **^{13}C NMR** (101 MHz, CDCl_3): δ 148.25, 120.33, 49.78, 32.26, 31.55, 25.31, 22.25, 19.65, 13.74, 13.39.

[0082] **HRMS** (ESI +) m/z : $[\text{C}_{10}\text{H}_{19}\text{N}_3 + \text{H}]^+$ calculated 182.16517, found 182.16539.

1.6. Synthesis of 4-butyl-1H-1,2,3-triazole-1-acetic acid ethyl ester (**3**)

[0083]



[0084] Synthesised from General Procedure A; **3** (1.99 g, 9.44 mmol, 56%) was obtained starting from sodium azide (25.5 mmol), ethyl bromoacetate (17.0 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.0 mmol), sodium ascorbate (6.0 mmol) and 1-hexyne (25.5 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 4:1; R_f = 0.15).

[0085] **Yield:** 56% (white solid).

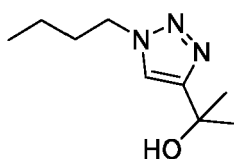
[0086] **^1H NMR** (400 MHz, CDCl_3): δ 7.38 (s, 1H), 5.07 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 2.68 (t, J = 7.7 Hz, 2H), 1.61 (p, J = 7.7 Hz, 2H), 1.33 (h, J = 7.3 Hz, 2H), 1.23 (t, J = 7.6 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H).

[0087] **^{13}C NMR** (101 MHz, CDCl_3): δ 166.48, 148.71, 121.97, 62.17, 50.69, 31.36, 25.24, 22.17, 13.97, 13.72.

[0088] **HRMS** (ESI +) m/z : $[\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_2 + \text{H}]^+$ calculated 212.13935, found 212.13977.

1.7 Synthesis of 1-butyl-4-(α,α -dimethyl methanol)-1H-1,2,3-triazole (**4**)

[0089]



[0090] Synthesised from General Procedure A; **4** (3.12 g, 17.0 mmol, 100%) was obtained starting from sodium azide (25.4 mmol), 1-bromobutane (17.0 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.0 mmol), sodium ascorbate (5.8 mmol) and 2-methyl-3-butyne-2-ol (25.5 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 1:1; R_f = 0.22).

[0091] **Yield:** quant. (yellow liquid).

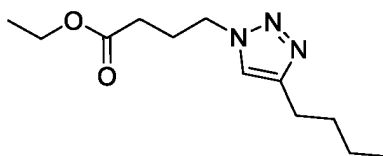
[0092] **^1H NMR** (400 MHz, CDCl_3): δ 7.43 (s, 1H), 4.31 (t, J = 7.3 Hz, 2H), 2.77 (s, 1H), 1.87 (p, J = 7.4 Hz, 2H), 1.62 (s, 6H), 1.35 (h, J = 7.4 Hz, 2H), 0.94 (t, J = 7.4 Hz, 3H).

[0093] **^{13}C NMR** (101 MHz, CDCl_3): δ 155.49, 118.90, 68.46, 50.04, 32.24, 30.46, 19.72, 13.43.

[0094] **HRMS** (ESI +) m/z : $[\text{C}_9\text{H}_{17}\text{N}_3\text{O} + \text{H}]^+$ calculated 184.14444, found 184.14458.

1.8 Synthesis of 4-butyl-1H-1,2,3-triazole-1-butanoic acid ethyl ester (**5**)

[0095]



[0096] Synthesised from General Procedure A; **5** (1.08 g, 4.5 mmol, 56%) was obtained starting from sodium azide (8.0 mmol), ethyl 4-bromobutyrate (8.4 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.7 mmol), sodium ascorbate (4 mmol) and 1-hexyne (8.0 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 3:1; R_f = 0.24).

[0097] Yield: 56% (pale yellow oil).

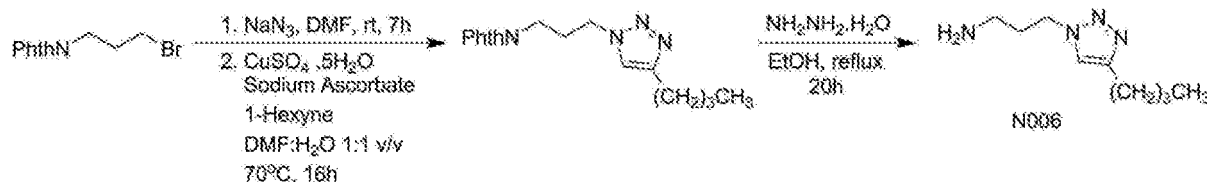
[0098] ^1H NMR (400 MHz, CDCl_3): δ 7.24 (s, 1H), 4.34 (t, J = 6.9 Hz, 2H), 4.08 (q, J = 7.1 Hz, 2H), 2.65 (t, J = 7.7 Hz, 2H), 2.28 (t, J = 7.1 Hz, 2H), 2.15 (p, J = 6.9 Hz, 2H), 1.59 (p, J = 7.6 Hz, 2H), 1.33 (h, J = 7.4 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H).

[0099] ^{13}C NMR (101 MHz, CDCl_3): δ 172.32, 148.43, 120.62, 60.60, 48.95, 31.50, 30.70, 25.46, 25.28, 22.24, 14.12, 13.75.

[0100] HRMS (ESI +) m/z : $[\text{C}_{12}\text{H}_{21}\text{O}_2\text{N}_3 + \text{H}]^+$ calculated 240.17065, found 240.17061.

1.9 Synthesis of substituted triazoles **6** - **8** via phthalimide-protected intermediates

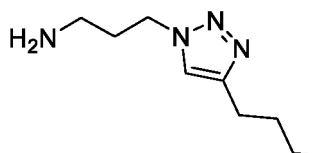
[0101]



Scheme 3: Reaction scheme for the synthesis of triazole **6**.

1.10 Synthesis of 4-butyl-1H-1,2,3-triazole-1-propanamine (**6**)

[0102]



[0103] Synthesised from modified reported procedures (Pyta, K., et al., European Journal of Medicinal Chemistry 2014, 84, 651; Wang, Y.-F., et al., Organic Letters 2013, 15(11), 2842). *N*-(3-Bromopropyl)phthalimide (10.1 mmol) and sodium azide (15.2 mmol) were dissolved in DMF (26 mL) under argon and stirred at room temperature for 7 hrs. The reaction was diluted with H_2O (26 mL) before the addition of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.0 mmol), sodium ascorbate (5.3 mmol) and 1-hexyne (25.5 mmol) in succession. The reaction was heated at 70°C with vigorous stirring.

[0104] The reaction was cooled to room temperature after 16 hours, before being diluted with H_2O (80 mL) and extracted with ethyl acetate (3 x 80 mL). The extracts were combined, concentrated and purified by silica chromatography (Pet. Ether/EtOAc, 2:3; R_f = 0.33). If crude failed to solidify due to remaining DMF, the sample was treated with 5% aq. LiCl solution to cause precipitation of 2-[3-(4-butyl-1H-1,2,3-triazol-1-yl)propyl]-1H-isoindole-1,3(2H)-dione as a cream powder (84%).

[0105] ^1H NMR (400 MHz, CDCl_3): δ 7.89 - 7.80 (m, 2H), 7.78 - 7.68 (m, 2H), 7.44 (s, 1H), 4.36 (t, J = 6.9 Hz, 2H), 3.74 (t, J = 6.5 Hz, 2H), 2.68 (t, J = 7.7 Hz, 2H), 2.31 (p, J = 6.8 Hz, 2H), 1.63 (p, J = 7.4 Hz, 2H), 1.37 (h, J = 7.4 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H).

[0106] ^{13}C NMR (101 MHz, CDCl_3): δ 168.29, 148.41, 134.17, 131.87, 123.36, 121.00, 47.58, 35.11, 31.53, 29.44, 25.33, 22.30, 13.81.

[0107] HRMS (ESI +) m/z : $[\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_2 + \text{H}]^+$ calculated 313.16590, found 313.16592.

[0108] 2-[3-(4-Butyl-1H-1,2,3-triazol-1-yl)propyl]-1H-isoindole-1,3(2H)-dione (8.5 mmol) was dissolved in ethanol (0.06 M) before being treated with hydrazine monohydrate (12.67 mmol). The solution was stirred vigorously and

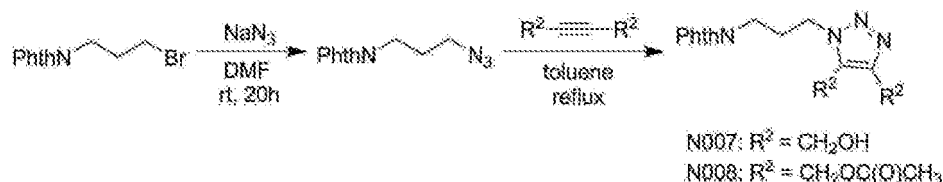
heated to 90°C. After heating overnight, a white precipitate had formed. The reaction was cooled, and the precipitate was removed by filtration and washed thoroughly. The filtrate was concentrated, and the resulting solid was resuspended in CH₂Cl₂ and filtered again. The filtrate was concentrated to a yellow oil which was purified by silica chromatography (CH₂Cl₂/MeOH/30% aq. NH₃, 10:1:0.1; R_f = 0.16) to give **6** as a cream solid (1.01 g, 5.5 mmol, 65%).

[0109] Yield: 65% (cream solid).

[0110] ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.80 (s, 1H), 4.33 (t, *J* = 7.0 Hz, 2H), 2.57 (t, *J* = 7.6 Hz, 2H), 2.47 (t, *J* = 6.6 Hz, 2H), 1.82 (p, *J* = 6.8 Hz, 2H), 1.64 - 1.44 (m, 4H), 1.29 (h, *J* = 7.3 Hz, 2H), 0.87 (t, *J* = 7.4 Hz, 3H).

[0111] ¹³C NMR (101 MHz, DMSO-*d*₆): δ 147.17, 122.06, 47.40, 38.88, 34.08, 31.60, 25.14, 22.12, 14.10.

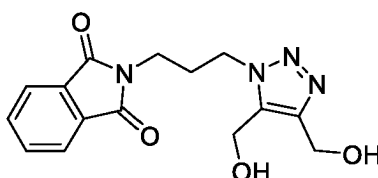
[0112] HRMS (ESI +) *m/z*: [C₉H₁₈N₄ + H]⁺ calculated 183.16042, found 183.16057.



Scheme 4: Reaction scheme for the synthesis of triazoles **7** and **8**.

1.11 Synthesis of 2-[3-[4,5-di(hydroxymethyl)-1H-1,2,3-triazole]propyl]-isoindoline-1,3-dione (**7**)

[0113]



[0114] Sodium azide (9.4 mmol) was suspended in DMF (26 mL) under argon, and to this solution *N*-(3-bromopropyl)phthalimide (8.7 mmol) was added. The mixture was stirred at room temperature overnight. The reaction was then diluted slowly with H₂O (100 mL), before extraction with ether. Concentration of the ethereal extracts provided *N*-(3-azidopropyl)phthalimide as a waxy cream solid (1.83 g, 7.94 mmol, 92%).

[0115] ¹H NMR (400 MHz, CDCl₃): δ 7.90 - 7.79 (m, 2H), 7.77 - 7.67 (m, 2H), 3.78 (t, *J* = 6.8 Hz, 2H), 3.38 (t, *J* = 6.7 Hz, 2H), 1.96 (p, *J* = 6.8 Hz, 2H).

[0116] ¹³C NMR (101 MHz, CDCl₃): δ 168.25, 134.04, 132.00, 123.31, 49.04, 35.38, 28.03.

[0117] HRMS (ESI +) *m/z*: [C₁₁H₁₀O₂N₄ + H]⁺ calculated 231.08765, found 231.08771.

[0118] *N*-(3-azidopropyl)phthalimide (7.9 mmol) was suspended in toluene (0.2 M) before addition of 2-butyne-1,4-diol (8.7 mmol). The reaction was stirred vigorously and heated to 115°C for 41 hrs. Toluene was evaporated and the crude solid was recrystallised from H₂O to give **7** as a white powder (1.34 g, 4.3 mmol, 54%).

[0119] Yield: 54% (white powder).

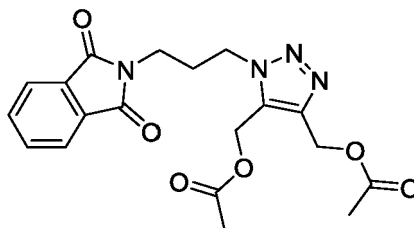
[0120] ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.89 - 7.77 (m, 4H), 5.29 (t, *J* = 5.4 Hz, 1H), 5.01 (t, *J* = 5.6 Hz, 1H), 4.57 (d, *J* = 5.3 Hz, 2H), 4.46 (d, *J* = 5.5 Hz, 2H), 4.37 (t, *J* = 7.3 Hz, 2H), 3.65 (t, *J* = 7.0 Hz, 2H), 2.18 (p, *J* = 7.2 Hz, 2H).

[0121] ¹³C NMR (101 MHz, DMSO-*d*₆): δ 168.36, 145.05, 134.76, 134.52, 132.16, 123.44, 54.63, 51.10, 45.99, 35.69, 28.88.

[0122] HRMS (ESI +) *m/z*: [C₁₅H₁₆O₄N₄ + H]⁺ calculated 317.12443, found 317.12448.

1.12 Synthesis of 2-[3-[4,5-(methyl ethanoate)-1H-1,2,3-triazole]propyl]-isoindoline-1,3-dione (**8**)

[0123]



[0124] As for **7**, using 2-butyne-1,4-diol diacetate as alkyne, and the crude product was recrystallised from ethanol to give **8** as white crystals (3.69 g, 9.23 mmol, 71%).

[0125] **Yield:** 71% (white crystals).

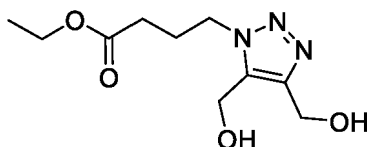
[0126] **¹H NMR** (400 MHz, CDCl₃): δ 7.89 - 7.80 (m, 2H), 7.78 - 7.69 (m, 2H), 5.23 (s, 2H), 5.22 (s, 2H), 4.46 - 4.37 (m, 2H), 3.82 (t, *J* = 6.7 Hz, 2H), 2.36 (p, *J* = 6.9 Hz, 2H), 2.06 (s, 3H), 2.04 (s, 3H).

[0127] **¹³C NMR** (101 MHz, CDCl₃): δ 170.67, 170.02, 168.20, 142.09, 134.17, 131.89, 130.65, 123.36, 56.67, 52.64, 46.51, 35.23, 29.09, 20.82, 20.50.

[0128] **HRMS** (ESI +) *m/z*: [C₁₉H₂₀N₄O₆ + H]⁺ calculated 401.14556, found 401.14563.

1.13 Synthesis of ethyl 4,5-bis(hydroxymethyl)-1H-1,2,3-triazole-1-butylate (**9**)

[0129]



[0130] Synthesised from modified General Procedure B; **9** (1.21 g, 4.6 mmol, 33%) was obtained starting from sodium azide (22 mmol) and ethyl 4-bromobutylate (19 mmol) heating in DMF (20 mL). The crude azide formed was treated with 2-butyne-1,4-diol (14 mmol) in toluene at 115°C for 24 hrs. The crude mixture was purified by silica chromatography (CH₂Cl₂/CH₃OH, 10:0.6; R_f = 0.28).

[0131] **Yield:** 33% (pale yellow oil).

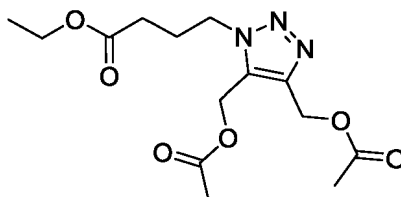
[0132] **¹H NMR** (400 MHz, CDCl₃): δ 4.94 (s, 2H), 4.67 (s, 2H), 4.58 (s, 2H), 4.37 (t, *J* = 7.1 Hz, 2H), 4.06 (q, *J* = 7.1 Hz, 2H), 2.32 (t, *J* = 7.1 Hz, 2H), 2.16 (p, *J* = 7.1 Hz, 2H), 1.20 (t, *J* = 7.1 Hz, 3H).

[0133] **¹³C NMR** (101 MHz, CDCl₃): δ 172.65, 144.67, 134.23, 60.73, 55.06, 51.82, 47.53, 30.77, 24.99, 14.10.

[0134] **HRMS** (ESI +) *m/z*: [C₁₀H₁₇N₃O₄ + H]⁺ calculated 244.12918, found 244.12921.

1.14. Synthesis of ethyl 4,5-bis(methyl ethanoate)-1H-1,2,3-triazole-1-butylate (**10**)

[0135]



[0136] Synthesised from General Procedure B; **10** (2.5 g, 7.7 mmol, 74%) was obtained from sodium azide (15.8 mmol), ethyl 4-bromobutylate (10.5 mmol) and 2-butyne-1,4-diol (11.1 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 3:2; R_f = 0.1).

[0137] **Yield:** 74% (colourless oil).

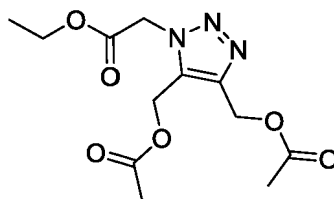
[0138] **¹H NMR** (400 MHz, CDCl₃): δ 5.24 (s, 2H), 5.23 (s, 2H), 4.42 (t, *J* = 7.1 Hz, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 2.39 (t, *J* = 7.0 Hz, 2H), 2.22 (p, *J* = 7.1 Hz, 2H), 2.06 (s, 3H), 2.05 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H).

[0139] **¹³C NMR** (101 MHz, CDCl₃): δ 172.24, 170.65, 170.01, 142.06, 130.64, 60.71, 56.71, 52.69, 47.65, 30.73, 25.15, 20.80, 20.56, 14.15.

[0140] **HRMS** (ESI +) *m/z*: [C₁₄H₂₁N₃O₆ + H]⁺ calculated 328.15031, found 328.15021.

1.15 Synthesis of ethyl 4,5-bis(methyl ethanoate)-1H-1,2,3-triazole-1-acetate (**11**)

[0141]



[0142] Synthesised from General Procedure B; **11** (1.9 g, 6.3 mmol, 63%) was obtained from sodium azide (15.1 mmol), ethyl-2-bromoacetate (10.0 mmol) and 2-butyne-1,4-diol diacetate (10.7 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 1:1; R_f = 0.43).

[0143] Yield: 63% (pale yellow oil).

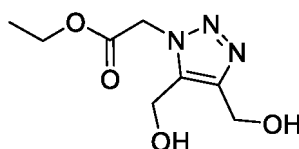
[0144] $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 5.25 (s, 2H), 5.24 (s, 2H), 5.23 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 2.06 (s, 3H), 2.03 (s, 3H), 1.29 (t, J = 7.1 Hz, 3H).

[0145] $^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ 170.68, 170.16, 166.23, 142.21, 131.79, 62.50, 56.60, 52.97, 49.78, 20.81, 20.48, 14.04.

[0146] HRMS (ESI +) m/z : $[\text{C}_{12}\text{H}_{17}\text{N}_3\text{O}_6 + \text{H}]^+$ calculated 300.11901, found 300.11890.

1.16. Synthesis of ethyl 4,5-bis(hydroxymethyl)-1H-1,2,3-triazole-1-acetate (**12**)

[0147]



[0148] Synthesised from a reported procedure (Wen, Y.-n., *et al.*, *Nucleosides, Nucleotides and Nucleic Acids* 2016, 35(3), 147). **12** (0.85 g, 3.9 mmol, 33%) was obtained from sodium azide (14.3 mmol), ethyl-2-bromoacetate (13.5 mmol) and 2-butyne-1,4-diol (12.1 mmol). The crude mixture was purified by silica chromatography ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$, 10:1; R_f = 0.13).

[0149] Yield: 33% (white solid).

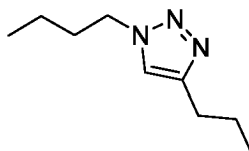
[0150] $^1\text{H NMR}$ (400 MHz, $\text{DMSO}-d_6$): δ 5.34 (t, J = 5.5 Hz, 1H), 5.31 (s, 2H), 5.08 (t, J = 5.7 Hz, 1H), 4.57 (d, J = 5.4 Hz, 2H), 4.50 (d, J = 5.5 Hz, 2H), 4.15 (q, J = 7.1 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H).

[0151] $^{13}\text{C NMR}$ (101 MHz, $\text{DMSO}-d_6$): δ 167.58, 144.81, 135.15, 61.82, 54.61, 51.67, 49.70, 14.40.

[0152] HRMS (ESI +) m/z : $[\text{C}_8\text{H}_{13}\text{N}_3\text{O}_4 + \text{H}]^+$ calculated 216.09788, found 216.09734.

1.17. Synthesis of 1-butyl-4-propyl-1H-1,2,3-triazole (**13**)

[0153]



[0154] Synthesised from General Procedure A; **13** (2.55 g, 15.2 mmol, 89%) was obtained starting from sodium azide (20.3 mmol), 1-bromobutane (17.0 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.0 mmol), sodium ascorbate (5.2 mmol) and 1-pentyne (20.0 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 3:2; R_f = 0.37).

[0155] Yield: 89% (colourless oil).

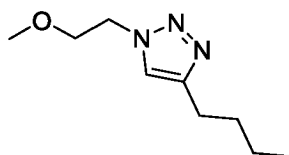
[0156] $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 7.23 (s, 1H), 4.28 (t, J = 7.2 Hz, 2H), 2.66 (t, J = 7.6 Hz, 2H), 1.84 (p, J = 7.3 Hz, 2H), 1.66 (h, J = 7.4 Hz, 2H), 1.32 (h, J = 7.4 Hz, 2H), 0.96 - 0.89 (m, 6H).

[0157] $^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ 148.11, 120.38, 49.82, 32.29, 27.66, 22.70, 19.68, 13.74, 13.43.

[0158] HRMS (ESI +) m/z : $[C_9H_{17}N_3 + H]^+$ calculated 168.14952, found 168.14951.

1.18 Synthesis of 1-(2-methoxyethyl)-4-butyl-1H-1,2,3-triazole (14)

[0159]



[0160] **14** (0.75 g, 4.1 mmol, 65%) was obtained from purified **15** (6.3 mmol) dissolved in dry THF (42 mL) under argon, cooled to 0°C. NaH (6.6 mmol) was added in a single portion. Once gas evolution had ceased, MeI (9.5 mmol) was added in three portions the mixture stirred at room temperature for 24 hours. The reaction was diluted with H₂O and THF was removed *in vacuo*. The product was extracted into ethyl acetate and concentrated. The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 1:1; R_f = 0.21).

[0161] **Yield**: 65% (colourless oil).

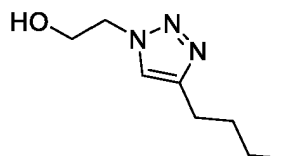
[0162] **¹H NMR** (400 MHz, CDCl₃): δ 7.36 (s, 1H), 4.45 (t, J = 5.0 Hz, 2H), 3.71 (t, J = 5.0 Hz, 2H), 3.32 (s, 3H), 2.68 (t, J = 7.8 Hz, 2H), 1.63 (p, J = 7.8 Hz, 2H), 1.36 (h, J = 7.3 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H).

[0163] **¹³C NMR** (101 MHz, CDCl₃): δ 148.26, 121.61, 70.91, 58.93, 50.06, 31.52, 25.31, 22.29, 13.78.

[0164] HRMS (ESI +) m/z : $[C_9H_{17}N_3O + H]^+$ calculated 184.14444, found 184.14445.

1.19 Synthesis of 4-propyl-1H-1,2,3-triazole-1-ethanol (15)

[0165]



[0166] Synthesised from General Procedure A; **15** (1.06 g, 6.3 mmol, 36%) was obtained starting from sodium azide (26.2 mmol), 2-bromoethanol (17.5 mmol), CuSO₄·5H₂O (1.0 mmol), sodium ascorbate (5.6 mmol) and 1-hexyne (25.5 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 2:3; R_f = 0.1).

[0167] **Yield**: 36% (pale yellow liquid).

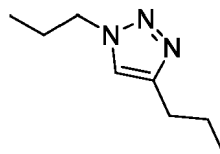
[0168] **¹H NMR** (400 MHz, CDCl₃): δ 7.41 (s, 1H), 4.70 (s, 1H), 4.37 (t, J = 5.2 Hz, 2H), 3.95 (t, J = 5.1 Hz, 2H), 2.62 - 2.53 (m, 2H), 1.54 (p, J = 7.4 Hz, 2H), 1.29 (h, J = 7.3 Hz, 2H), 0.85 (t, J = 7.4 Hz, 3H).

[0169] **¹³C NMR** (101 MHz, CDCl₃): δ 147.87, 121.99, 60.71, 52.62, 31.36, 25.11, 22.20, 13.73.

[0170] HRMS (ESI +) m/z : $[C_8H_{15}N_3O + H]^+$ calculated 170.12879, found 170.12887.

1.20. Synthesis of 1,4-dipropyl-1H-1,2,3-triazole (16)

[0171]



[0172] Synthesised from General Procedure A, **16** (0.68 g, 4.36 mmol, 26%) was obtained starting from sodium azide (20.0 mmol), 1-bromopropane (17.0 mmol), CuSO₄·5H₂O (1.0 mmol), sodium ascorbate (5.1 mmol) and 1-pentyne (20.1 mmol). The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 2:1; R_f = 0.23).

[0173] **Yield**: 26% (colourless oil).

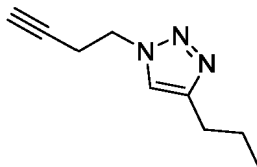
[0174] **¹H NMR** (400 MHz, CDCl₃): δ 7.24 (s, 1H), 4.25 (t, J = 7.2 Hz, 2H), 2.66 (t, J = 7.5 Hz, 2H), 1.89 (h, J = 7.3 Hz, 2H), 1.66 (h, J = 7.4 Hz, 2H), 0.96 - 0.89 (m, 6H).

[0175] ^{13}C NMR (101 MHz, CDCl_3): δ 148.10, 120.46, 51.71, 27.64, 23.72, 22.70, 13.74, 11.04.

[0176] HRMS (ESI +) m/z : $[\text{C}_8\text{H}_{15}\text{N}_3 + \text{H}]^+$ calculated 154.13387, found 154.13385.

1.21. Synthesis of 1-(3-butyn-1-yl)-4-propyl-1H-1,2,3-triazole (**17**)

[0177]



[0178] Synthesised from a modified General Procedure A. **17** (0.30g, 1.8 mmol, 18%) was obtained from sodium azide (15.0 mmol) and 1-bromobutyn-1-yl (10.0 mmol) heating at 60°C in DMF (15 mL) for 3 hours. The reaction was cooled and diluted with H_2O (15 mL), followed by addition of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.2 mmol), sodium ascorbate (2.0 mmol) and 1-pentyne (17.9 mmol). The reaction was stirred at room temperature overnight. The crude mixture was purified by silica chromatography (Pet. Ether/Diethyl Ether, 2:1; R_f = 0.17).

[0179] Yield: 18% (pale yellow oil).

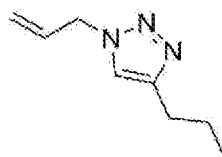
[0180] ^1H NMR (400 MHz, CDCl_3): δ 7.39 (s, 1H), 4.46 (t, J = 6.7 Hz, 2H), 2.76 (td, J = 6.7, 2.6 Hz, 2H), 2.68 (t, J = 7.6 Hz, 2H), 2.05 (t, J = 2.6 Hz, 1H), 1.68 (h, J = 7.4 Hz, 2H), 0.95 (t, J = 7.4 Hz, 3H).

[0181] ^{13}C NMR (101 MHz, CDCl_3): δ 148.14, 121.10, 79.69, 71.32, 48.53, 27.60, 22.66, 20.65, 13.72.

[0182] HRMS (ESI +) m/z : $[\text{C}_9\text{H}_{13}\text{N}_3 + \text{H}]^+$ calculated 164.11822, found 164.11824.

1.22. Synthesis of 1-(2-propen-1-yl)-4-propyl-1H-1,2,3-triazole (**18**)

[0183]



[0184] Synthesised from a modified General Procedure A. **18** (1.77 g, 11.7 mmol, 78%) was obtained from sodium azide (21.0 mmol), allyl bromide (15.0 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.9 mmol), sodium ascorbate (4.5 mmol) and 1-pentyne (21.0 mmol), heated overnight at 45°C . The crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 4:1; R_f = 0.13).

[0185] Yield: 78% (colourless oil).

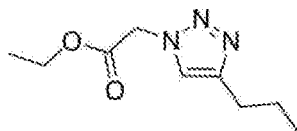
[0186] ^1H NMR (400 MHz, CDCl_3): δ 7.26 (s, 1H), 6.08 - 5.93 (m, 1H), 5.37 - 5.27 (m, 1H), 5.33 - 5.20 (m, 1H), 4.94 (dt, J = 6.1, 1.4 Hz, 2H), 2.69 (t, J = 7.6 Hz, 2H), 1.69 (h, J = 7.4 Hz, 2H), 0.96 (t, J = 7.3 Hz, 3H).

[0187] ^{13}C NMR (101 MHz, CDCl_3): δ 148.50, 131.58, 120.39, 119.73, 52.54, 27.69, 22.70, 13.75.

[0188] HRMS (ESI +) m/z : $[\text{C}_8\text{H}_{13}\text{N}_3 + \text{H}]^+$ calculated 152.1182, found 152.1183.

1.23. Synthesis of ethyl 2-(4-propyl-1H-1,2,3-triazol-1-yl)-acetate (**19**)

[0189]



[0190] Synthesised from General Procedure A; **19** (2.83 g, 14.4 mmol, 85%) was obtained from sodium azide (20 mmol), ethyl bromoacetate (17.1 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.0 mmol), sodium ascorbate (5.1 mmol) and 1-pentyne (20 mmol), heated at 50°C for 9 hrs. Following workup, the crude mixture was purified by silica chromatography (Pet. Ether/EtOAc, 1:1; R_f = 0.27).

Yield 85% (cream waxy solid)

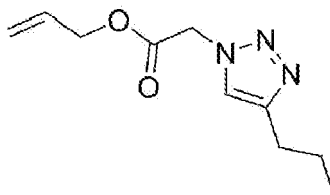
¹H NMR (400 MHz, CDCl₃): δ 7.41 (s, 1H), 5.11 (s, 2H), 4.24 (q, *J* = 7.2 Hz, 2H), 2.71 (t, *J* = 7.6 Hz, 2H), 1.70 (h, *J* = 7.4 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 0.96 (t, *J* = 7.4 Hz, 3H).

[0191] **¹³C NMR** (101 MHz, CDCl₃): δ 166.44, 148.62, 122.02, 62.28, 50.77, 27.58, 22.55, 14.03, 13.69.

[0192] **HRMS** (ESI +) *m/z*: [C₉H₁₅N₃O₂ + H]⁺ calculated 198.12370, found 198.12385.

1.24. Synthesis of prop-2-en-1-yl 2-(4-propyl-1H-1,2,3-triazol-1-yl)-acetate (**20**)

[0193]



[0194] **19** was saponified to the corresponding acid following the procedure reported in Sabbah *et al.* (Sabbah, M., et al., Bioorganic & Medicinal Chemistry, 2012, 20(15), 4727-4736) with ethanolic KOH. The resulting acid (1.9 mmol), 4-dimethylaminopyridine (0.2 mmol) and allyl alcohol (4.4 mmol) were combined in dichloromethane (15 mL) under argon. After stirring for 30 minutes, the solution was cooled to 0°C before adding *N,N'*-dicyclohexylcarbodiimide (2 mmol). The solution was stirred at room temperature for 24 hours and then filtered and concentrated *in vacuo*. The residue was resuspended in ethyl acetate and filtered again. The filtrate was concentrated to an oil, which was purified by silica chromatography (Pet. Ether/EtOAc, 1:1, *R*_f = 0.33).

Yield 70% (white waxy solid)

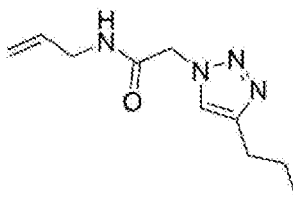
¹H NMR (400 MHz, CDCl₃): δ 7.42 (s, 1H), 5.97 - 5.82 (m, 1H), 5.38 - 5.24 (m, 2H), 5.15 (s, 2H), 4.72 - 4.64 (m, 2H), 2.72 (t, *J* = 7.6 Hz, 2H), 1.71 (h, *J* = 7.3 Hz, 2H), 0.97 (t, *J* = 7.3 Hz, 3H).

[0195] **¹³C NMR** (101 MHz, CDCl₃): δ 166.15, 148.67, 130.83, 122.02, 119.62, 66.68, 50.72, 27.58, 22.56, 13.71.

[0196] **HRMS** (ESI +) *m/z*: [C₁₀H₁₅N₃O₂ + H]⁺ calculated 210.12370, found 210.12392.

1.25. Synthesis of prop-2-en-1-yl 2-(4-propyl-1H-1,2,3-triazol-1-yl)-acetamide (**21**)

[0197]



[0198] **19** was saponified to the corresponding acid following the procedure reported in Sabbah *et al.* (Sabbah, M., et al., Bioorganic & Medicinal Chemistry, 2012, 20(15), 4727-4736) with ethanolic KOH. The resulting acid (1.8 mmol) was dissolved in dichloromethane/dimethylformamide (20 mL/2.5 mL) under argon, and treated with HOBT (4.0 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (4.0 mmol) and allyl amine (4.0 mmol). After stirring for 10 hrs at room temperature, the mixture was treated with 5 drops of acetic acid and washed with H₂O and brine. The crude product was purified by silica chromatography (CH₂Cl₂/MeOH, 100:1 to 100:5 gradient, *R*_f = 0.1).

[0199] **Yield** 56% (cream solid).

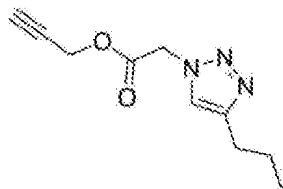
[0200] **¹H NMR** (400 MHz, CDCl₃): δ 7.46 (s, 1H), 6.43 (s, 1H), 5.83 - 5.69 (m, 1H), 5.14 - 5.07 (m, 2H), 5.05 (s, 2H), 3.91 - 3.82 (m, 2H), 2.71 (t, *J* = 7.6 Hz, 2H), 1.71 (h, *J* = 7.4 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H).

[0201] **¹³C NMR** (101 MHz, CDCl₃): δ 165.15, 148.87, 133.01, 122.53, 116.89, 53.07, 42.00, 27.47, 22.49, 13.72.

[0202] HRMS (ESI +) m/z : $[C_{10}H_{16}N_4O + H]^+$ calculated 209.13969, found 209.13990.

1.26. Synthesis of prop-2-yn-1-yl 2-(4-propyl-1H-1,2,3-triazol-1-yl)-acetate (22)

[0203]



[0204] **19** was saponified to the corresponding acid following the procedure reported in Sabbah *et al.* (Sabbah, M., et al., Bioorganic & Medicinal Chemistry, 2012, 20(15), 4727-4736) with ethanolic KOH. The resulting acid (1.8 mmol), 4-dimethylaminopyridine (0.4 mmol) and propargyl alcohol (3.4 mmol) were combined in dichloromethane (15 mL) under argon. After stirring for 30 minutes, the solution was cooled to 0°C and *N,N'*-dicyclohexylcarbodiimide (1.9 mmol) was added. The mixture was stirred at room temperature for 24 hours, filtered and concentrated *in vacuo*. The residue was resuspended in ethyl acetate and filtered again. The filtrate was concentrated to an oil, which was purified by silica chromatography (Pet. Ether/EtOAc, 1:1, R_f = 0.33).

Yield 71% (colourless liquid)

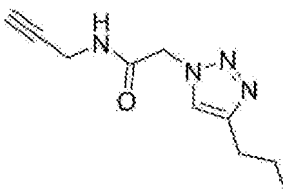
^1H NMR (400 MHz, $CDCl_3$): δ 7.43 (s, 1H), 5.19 (s, 2H), 4.79 (d, J = 2.5 Hz, 2H), 2.72 (t, J = 7.6 Hz, 2H), 2.53 (t, J = 2.4 Hz, 1H), 1.71 (h, J = 7.4 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H).

[0205] **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.77, 148.74, 122.06, 76.30, 76.07, 53.45, 50.52, 27.55, 22.53, 13.71.

[0206] HRMS (ESI +) m/z : $[C_{10}H_{13}N_3O_2 + H]^+$ calculated 208.10805, found 208.10828.

1.27. Synthesis of prop-2-yn-1-yl 2-(4-propyl-1H-1,2,3-triazol-1-yl)-acetamide (23)

[0207]



[0208] **19** was saponified to the corresponding acid following the procedure reported in Sabbah *et al.* (Sabbah, M., et al., Bioorganic & Medicinal Chemistry, 2012, 20(15), 4727-4736) with ethanolic KOH. The resulting acid (1.8 mmol) was dissolved in dichloromethane/dimethylformamide (20 mL/2.5 mL) under argon, and treated with HOBt (3.7 mmol), EDCI (4.0 mmol) and allyl amine (3.5 mmol). After stirring for 10 hrs at room temperature, the mixture was treated with 5 drops of acetic acid and washed with H_2O and brine. The crude product was purified by silica chromatography (CH_2Cl_2 /MeOH, 100:1 to 100:5 gradient, R_f = 0.1).

Yield 61% (cream solid)

^1H NMR (400 MHz, $CDCl_3$): δ 7.47 (s, 1H), 6.79 - 6.71 (m, 1H), 5.06 (s, 2H), 4.04 (dd, J = 5.4, 2.5 Hz, 2H), 2.71 (t, J = 7.6 Hz, 2H), 2.21 (t, J = 2.6 Hz, 1H), 1.71 (h, J = 7.4 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H).

[0209] **^{13}C NMR** (101 MHz, $CDCl_3$): δ 165.04, 148.87, 122.60, 78.45, 72.03, 52.84, 29.38, 27.47, 22.48, 13.73.

[0210] HRMS (ESI +) m/z : $[C_{10}H_{14}N_4O + H]^+$ calculated 207.12404, found 207.12411.

2. Soil Experiments

[0211] The soil used in this study was collected from four different locations in Victoria, Australia: (i) a wheat cropping soil from Horsham (36°45' S, 142°07' E), (ii) a rotational cropping soil from Dahlen (36°37' S, 142°09' E), (iii) a vegetable growing soil from Clyde (38°08' S, 145°20' E), and (iv) a pasture soil from Terang (38°15' S, 142°52' E). In addition, a sugarcane cropping soil from South Johnstone in northern Queensland (17°34' S, 145°57' E) was also studied. The water content of the soil was calculated before commencing each experiment, from samples that were oven-dried to constant weight. The soil's water-filled pore space (WFPS) was in the range 52%-61%, which is within the recommended 50-70% range for microbial activity due to oxygen and nutrient availability (Fichtner, T., et al., Applied Sciences, 2019, 9, 496).

[0212] The pH values and residual (initial) concentrations of ammonium-N and nitrate-N in the tested soils are compiled in Table 1 below.

[0213] 3,4-Dimethylpyrazole phosphate (DMPP), prepared as a solution of 3,4-dimethylpyrazole in phosphoric acid, was obtained from Incitec Pivot Fertilisers.

Table 1. Residual concentrations of ammonium-N and nitrate-N and pH of the soils tested in this study

Soil Location	Baseline (mg kg ⁻¹)		Organic Carbon (%)	pH (1:5 water)
	NH ₄ ⁺ -N	NO ₃ ⁻ -N		
South Johnstone	14	15	1.21	5.0
Terang	29	27	4.6	6.5
Clyde	2	48	1.9	7.2
Dahlen	3.3	270	1.02	7.3
Horsham	0.95	7.2	0.73	8.8

2.1. Soil Incubation Experiments

[0214] Soil microcosm incubations were carried out in 250 ml polypropylene specimen containers (Sarstedt, Germany), containing 18.24 g oven dry-weight equivalent of soil. Microcosms were re-wetted and pre-incubated at the test temperature for seven days to revive soil microbial activity. Following pre-incubation, the remaining volume to reach the desired water-filled pore spaces (WFPS%) was applied as one of the following treatment solutions; (NH₄)₂SO₄ (*Control*), (NH₄)₂SO₄ + DMPP, or (NH₄)₂SO₄ + one of Compounds 1-23. Each treatment was applied in triplicate per soil type, so that *n* = 3 at each time point.

[0215] Treatment solutions were prepared such that each microcosm received (NH₄)₂SO₄ at a rate of 100 mg N per kg soil, Compounds **1-23** at 10 mol % of applied N, or DMPP at one of 1.5, 3.6 or 10 mol % of applied N, referred to as L-DMPP, M-DMPP or H-DMPP respectively.

[0216] The microcosms were incubated for 0, 3, 7, 14, 21 or 28 days, where day 0 samples were extracted following 1-hour incubation post-treatment. Soil microcosms were aerated and moisture levels were replenished based on weight loss every few days throughout the incubation period.

[0217] At the end of each incubation period, soil microcosms were destructively sampled by treatment with 2M KCl (100 mL). After shaking for 1 hour, soil-KCl solutions were filtered (Whatman 42) before storing the filtrates at -20°C until the conclusion of the experiment. All KCl extracts were then analysed by Segmented Flow Analysis (San ++, Skalar, Breda, The Netherlands) for the concentration of nitrogen from ammonium (NH₄⁺-N) and nitrogen from NO₃⁻ and NO₂⁻ (NO_x⁻-N) after appropriate dilutions. Results are reported as the mean of three replicates, errors reported are standard errors of the mean.

2.1.2. Calculating Percent Nitrification and Percent Nitrification Inhibition

[0218] Nitrification calculations were performed as previously reported (Aulakh, M., et al., Biology and Fertility of Soils 2001, 33, 258-263; Mahmood, T., et al., Soil Research 2017, 55, 715-722) without correction for the initial baseline concentrations of NH₄⁺-N or NO₃⁻-N in the untreated soil. For each treatment nitrified NH₄⁺-N (%) was calculated according to eqn. 1:

$$\text{nitrified NH}_4^+\text{-N (\%)} = \frac{[\text{NH}_4^+\text{-N}]_0 - [\text{NH}_4^+\text{-N}]_t}{[\text{NH}_4^+\text{-N}]_0} \times 100 \quad (\text{eqn. 1})$$

where $[\text{NH}_4^+\text{-N}]_0$ is the $\text{NH}_4^+\text{-N}$ concentration (in mg N kg^{-1} soil) of the soil on day 0 and $[\text{NH}_4^+\text{-N}]_t$ is the $\text{NH}_4^+\text{-N}$ concentration (in mg N kg^{-1} soil) of the soil at a given time point t .

[0219] $\text{NO}_x^-\text{-N}$ accumulation rates ($\text{mg NO}_x^-\text{-N/kg soil/day}$) over the 28-day incubation experiments were calculated for each treatment as in the following (eqn. 2):

$$\text{NO}_x^-\text{-N accumulation} = \frac{[\text{NO}_x^-\text{-N}]_{t=28} - [\text{NO}_x^-\text{-N}]_{t=0}}{28} \quad (\text{eqn. 2})$$

$[\text{NO}_x^-\text{-N}]_{t=0}$ and $[\text{NO}_x^-\text{-N}]_{t=28}$ are the combined concentrations of nitrite (NO_2^-) and nitrate (NO_3^-) in the soil (in mg N kg^{-1} soil) on day 0 and day 28, respectively.

[0220] Nitrification inhibition (%) was calculated based on either $\text{NH}_4^+\text{-N}$ data (i.e., the percent nitrified $\text{NH}_4^+\text{-N}$ calculated from eqn. 1), or on $\text{NO}_x^-\text{-N}$ data. For nitrification inhibition based on $\text{NH}_4^+\text{-N}$, percent values were calculated from the nitrified $\text{NH}_4^+\text{-N}$ percentage of the fertilised control (only $(\text{NH}_4)_2\text{SO}_4$) at a given time point t , and the nitrified $\text{NH}_4^+\text{-N}$ percentage in the treated sample ($(\text{NH}_4)_2\text{SO}_4$ and NI) at the same time point, according to eqn. 3:

$$\text{nitrification inhibition (\%)}_{\text{based on NH}_4^+\text{-N}} = \frac{[\text{nitrified NH}_4^+\text{-N (\%)}]_{t, \text{control}} - [\text{nitrified NH}_4^+\text{-N (\%)}]_{t, \text{treated}}}{[\text{nitrified NH}_4^+\text{-N (\%)}]_{t, \text{control}}} \times 100 \quad (\text{eqn. 3})$$

[0221] For nitrification inhibition based on $\text{NO}_x^-\text{-N}$ concentrations, percent values were calculated from the $\text{NO}_x^-\text{-N}$ concentrations in the fertilised control (only $(\text{NH}_4)_2\text{SO}_4$) at a given time point, t , and the $\text{NO}_x^-\text{-N}$ concentrations in the treated sample ($(\text{NH}_4)_2\text{SO}_4$ and NI) at the same timepoint, according to eqn. 4:

$$\frac{[\text{NO}_x^-\text{-N}]_{t, \text{control}} - [\text{NO}_x^-\text{-N}]_{t, \text{treated}}}{[\text{NO}_x^-\text{-N}]_{t, \text{control}}} \times 100 \quad (\text{eqn. 4})$$

2.1.3 Statistical Analysis

[0222] All data presented are means of three replicates. Statistical analyses were performed on raw $\text{NH}_4^+\text{-N}$ and $\text{NO}_x^-\text{-N}$ data in R (version 3.5.2; R Core Team, 2018), using the statistical package *emmeans* (Lenth, 2019). Data were assessed for statistical significance ($P < .05$) via two-way analysis of variation (ANOVA; Chambers & Hastie, 1992) assessing the impact of the two factors "Day" and "Treatment", and pair-wise comparisons between treatments at each time point were evaluated using a TukeyHSD post-hoc adjustment. Statistical results for inhibitor treatments compared to both the fertilised control $(\text{NH}_4)_2\text{SO}_4$ treatment and DMPP treatment are illustrated in the tables displaying raw $\text{NH}_4^+\text{-N}$ and $\text{NO}_x^-\text{-N}$ data.

2.1.4. $\text{NH}_4^+\text{-N}$ and $\text{NO}_x^-\text{-N}$ Concentrations

[0223] Incubation tests of compounds compared to either low-concentration DMPP (1.5 mol%, L-DMPP) or medium-concentration DMPP (3.6 mol%, M-DMPP) treatments were conducted in all soils to obtain initial structure-activity relationship information to guide future synthesis. Selected compounds were re-tested along with Compounds **13** to **17** in the alkaline soils (Horsham, Dahlen) against high-concentration DMPP (10 mol%, H-DMPP) treatment. In most studies, the applied fertiliser $\text{NH}_4^+\text{-N}$ had been completely consumed in the control $(\text{NH}_4)_2\text{SO}_4$ treatments within the 28 days. In general, the nitrification inhibitors were most effective at slowing $\text{NO}_x^-\text{-N}$ production in the more alkaline soils (Horsham, Dahlen).

Terang Soil

[0224] In the acidic Terang soil, Compound **3** and L-DMPP were the most effective treatments at retaining more $\text{NH}_4^+\text{-N}$ in the soil than the fertilised control (see Table 2 below). Reduced $\text{NO}_x^-\text{-N}$ production was also observed for these treatments when compared to the fertilised control, predominantly until day 14 after which the concentrations

converged to that of the control.

Table 2. Ammonia nitrified (%) during a 28-day incubation at 25°C in Terang soil (pH 6.5). Emboldened values indicate nitrification rates lower than those observed in the control treatment ((NH₄)₂SO₄), correlating positively to inhibitor activity. Errors stated are standard errors of the mean, n = 3.

Treatment	Nitrified NH ₄ ⁺ -N (%)			
	Day 3	Day 7	Day 14	Day 28
(NH ₄) ₂ SO ₄	36.8 ± 7.4	72.7 ± 8.0	74.3 ± 7.4	94.3 ± 9.2
(NH ₄) ₂ SO ₄ + 1	13.6 ± 3.1	43.1 ± 3.7	85.1 ± 4.5	100 ± 4.2
(NH ₄) ₂ SO ₄ + 2	27.2 ± 2.0	64.8 ± 2.3	95.6 ± 2.8	100 ± 2.7
(NH ₄) ₂ SO ₄ + 3	15.6 ± 3.0	29.4 ± 2.6	47.8 ± 3.5	99.9 ± 2.8
(NH ₄) ₂ SO ₄ + 4	27.0 ± 7.5	67.3 ± 5.2	96.4 ± 5.8	100 ± 5.9
(NH ₄) ₂ SO ₄ + L-DMPP	23.5 ± 3.7	47.8 ± 2.0	89.6 ± 3.1	100 ± 2.5

Horsham Soil

[0225] As is evident from Figure 1 and Table 3, of the various inhibitors tested at 25°C, Compounds **13**, **16**, **17** (and to a lesser extent Compound **2**) performed statistically better at retaining NH₄⁺ than the uninhibited control treatment after 28 days with $P < .001$ for these compounds, except for Compound **2**, and inhibiting NO_x⁻ formation ($P = .013$ (**13**), $.001$ (**16**), $< .001$ (**17**)). At 35°C, all of Compounds **2**, **13**, **16** and **17** showed lower nitrification rates than the uninhibited control treatment. Of these, Compound **13** and DMPP performed statistically better at retaining NH₄⁺ ($P = .004$ (**13**) and $.008$ (DMPP)) and preventing NO_x⁻ production ($P = .03$ for both treatments), with Compound **13** being slightly more efficient in retarding nitrification of ammonia than DMPP (61% vs 65% NH₄⁺-N consumption, respectively).

Table 3. Ammonia nitrified (%) during a 28-day incubation at either 25°C or 35°C in Horsham soil (pH 8.8). Emboldened values indicate nitrification rates lower than those observed in the control treatment ((NH₄)₂SO₄), correlating positively to inhibitor activity. Errors stated are standard errors of the mean, n = 3.

Test Temp (°C)	Treatment	Nitrified NH ₄ ⁺ -N (%)				
		Day 3	Day 7	Day 14	Day 21	Day 28
25	(NH ₄) ₂ SO ₄	13.5 ± 8.4	68.5 ± 2.4	100 ± 2.7	100 ± 2.7	100 ± 2.7
	(NH ₄) ₂ SO ₄ + 2	12.2 ± 8.6	17.9 ± 6.9	44.5 ± 7.2	66.6 ± 8.9	90.4 ± 9.7
	(NH ₄) ₂ SO ₄ + 13	17.7 ± 3.9	23.4 ± 5.4	40.0 ± 4.3	47.2 ± 4.3	72.2 ± 4.8
	(NH ₄) ₂ SO ₄ + 14	23.8 ± 3.0	38.7 ± 5.3	84.3 ± 5.6	93.4 ± 6.2	100 ± 3.2
	(NH ₄) ₂ SO ₄ + 16	8.9 ± 3.4	24.9 ± 3.0	44.2 ± 3.3	57.5 ± 3.4	70.9 ± 3.9
	(NH ₄) ₂ SO ₄ + 17	13.5 ± 4.7	30.6 ± 5.5	35.1 ± 5.1	43.9 ± 5.7	65.8 ± 5.2
	(NH ₄) ₂ SO ₄ + H-DMPP	-6.9 ± 2.8	8.5 ± 2.8	37.2 ± 2.3	75.7 ± 5.4	76.2 ± 2.1
35	(NH ₄) ₂ SO ₄	18.8 ± 2.4	45.9 ± 1.7	81.6 ± 3.7	84.5 ± 11.0	98.0 ± 2.0
	(NH ₄) ₂ SO ₄ + 2	-3.3 ± 5.9	8.8 ± 5.5	40.3 ± 5.4	53.8 ± 9.4	71.6 ± 22.9
	(NH ₄) ₂ SO ₄ + 13	3.5 ± 6.4	18.7 ± 5.5	48.0 ± 5.3	59.3 ± 10.3	61.2 ± 14.2
	(NH ₄) ₂ SO ₄ + 14	13.2 ± 5.4	15.7 ± 5.7	39.8 ± 6.6	83.5 ± 7.2	79.7 ± 9.4
	(NH ₄) ₂ SO ₄ + 16	2.5 ± 4.5	22.3 ± 4.3	42.3 ± 4.6	74.9 ± 6.1	83.7 ± 6.1
	(NH ₄) ₂ SO ₄ + 17	-0.8 ± 3.4	8.5 ± 2.8	37.2 ± 2.3	75.7 ± 5.4	76.2 ± 2.1
	(NH ₄) ₂ SO ₄ + H-DMPP	7.9 ± 3.0	18.9 ± 2.8	32.3 ± 4.1	46.6 ± 5.9	64.5 ± 12.7

[0226] The calculated NO_x⁻-N production rates shown in Figure 2 indicate that incubation at 25°C led to lower NO_x⁻-N accumulation in all treatments compared with those at 35°C, except for Compounds **2** and **14**, where the NO_x⁻-N accumulation was lower at the elevated temperature. The rate of NO_x⁻-N accumulation in soil treated with Compound **13** was the same at both temperatures (2.8 mg NO_x⁻-N/kg soil/day), whilst treatment with H-DMPP showed the greatest increase in production rate at the higher test temperature.

Dahlen Soil

[0227] In the Dahlen soil, all of Compounds **2**, **13**, **16** and **17** performed statistically better ($P < .001$) at retaining $\text{NH}_4^+\text{-N}$ than the uninhibited control treatment and DMPP after 28 days at 25°C. The results from these tests are shown in Figure 3 and Table 4. At the elevated temperature of 35°C, all four triazoles out-performed H-DMPP at slowing the rate of ammonia nitrification. The considerably large error for the $\text{NO}_x\text{-N}$ measurements shown in Figures 3B and 3D is likely due to the fact that this soil was particularly rich in NO_3^- ($\text{NO}_3^-\text{-N}$: 270 mg kg^{-1}), compared with the other soils (Horsham $\text{NO}_3^-\text{-N}$: 7.2 mg kg^{-1} ; Terang $\text{NO}_3^-\text{-N}$: 27 mg kg^{-1}) prior to commencing testing.

[0228] Incubation studies in this soil at 35°C with DMPP and Compounds **2**, **13**, **16** and **17** (data are included in Table 4) revealed that DMPP performed significantly poorer at the higher temperature, while all of Compounds **2**, **13**, **16** and **17** performed statistically better ($P < .001$) at retaining NH_4^+ than both the DMPP treatment and control treatment after 28 days, with ammonia consumption ranging from 17% (**16**) to 38% (**17**). The measured concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_x\text{-N}$ for these compounds are shown in Figure 3C and 3D.

[0229] The rate of $\text{NO}_x\text{-N}$ accumulation in the soil over the 28-day incubation period is shown in Figure 4. Thus, incubation at 25°C resulted in higher $\text{NO}_x\text{-N}$ accumulation for all treatments compared with those performed at 35°C, except for DMPP. Treatment with **16** at 35°C resulted in the lowest accumulation rate (1.8 $\text{mg NO}_x\text{-N/kg soil/day}$), whereas the highest accumulation rate in a treated soil occurred for treatment with Compound **17** at 25°C (4.7 $\text{mg NO}_x\text{-N/kg soil/day}$). Interestingly, the accumulation rate dropped to 2.4 $\text{mg NO}_x\text{-N/kg soil/day}$ for Compound **17** at 35°C, which is the largest reduction in the accumulation rate for all inhibitors tested in this series. On the other hand, the rate of $\text{NO}_x\text{-N}$ accumulation in soil treated with Compound **13** was least affected by the temperature change (2.5 vs 2.4 $\text{mg NO}_x\text{-N/kg soil/day}$, at 25°C and 35°C, respectively), mirroring the seemingly temperature-independent behaviour observed in the Horsham soil for this Compound.

Table 4. Ammonia nitrified (%) during a 28-day incubation at either 25°C or 35°C in Dahlen soil (pH 7.3). Emboldened values indicate nitrification rates lower than those observed in the control treatment ($(\text{NH}_4)_2\text{SO}_4$), correlating positively to inhibitor activity. Errors stated are standard errors of the mean, $n = 3$.

Test Temp (°C)	Treatment	Nitrified $\text{NH}_4^+\text{-N}$ (%)				
		Day 3	Day 7	Day 14	Day 21	Day 28
25	$(\text{NH}_4)_2\text{SO}_4$	7.3 ± 3.1	19.6 ± 15.2	41.1 ± 10.0	52.0 ± 9.5	83.6 ± 13.3
	$(\text{NH}_4)_2\text{SO}_4$ + 2	7.5 ± 5.2	21.2 ± 12.2	20.3 ± 21.6	32.9 ± 18.4	37.6 ± 11.0
	$(\text{NH}_4)_2\text{SO}_4$ + 13	10.6 ± 3.7	22.2 ± 50.2	24.9 ± 59.3	32.6 ± 2.4	41.5 ± 1.2
	$(\text{NH}_4)_2\text{SO}_4$ + 16	8.8 ± 3.3	15.6 ± 5.0	16.4 ± 28.7	26.9 ± 4.6	37.3 ± 4.4
	$(\text{NH}_4)_2\text{SO}_4$ + 17	3.0 ± 1.3	10.4 ± 3.6	12.1 ± 3.2	22.2 ± 5.0	36.4 ± 6.8
	$(\text{NH}_4)_2\text{SO}_4$ + H-DMPP	2.1 ± 2.8	11.7 ± 6.7	17.3 ± 12.7	18.2 ± 6.6	18.6 ± 4.9
35	$(\text{NH}_4)_2\text{SO}_4$	4.0 ± 4.7	17.5 ± 4.3	36.1 ± 4.7	56.9 ± 5.0	58.3 ± 9.3
	$(\text{NH}_4)_2\text{SO}_4$ + 2	1.7 ± 3.3	-1.3 ± 1.9	2.0 ± 2.4	29.6 ± 3.6	22.4 ± 1.5
	$(\text{NH}_4)_2\text{SO}_4$ + 13	-1.2 ± 1.1	3.5 ± 1.3	9.2 ± 1.1	34.2 ± 2.4	33.6 ± 1.3
	$(\text{NH}_4)_2\text{SO}_4$ + 16	1.5 ± 1.4	6.0 ± 1.5	16.1 ± 4.5	29.8 ± 1.2	16.9 ± 2.6
	$(\text{NH}_4)_2\text{SO}_4$ + 17	6.3 ± 2.2	8.8 ± 1.9	17.7 ± 2.6	38.0 ± 2.8	37.6 ± 3.1
	$(\text{NH}_4)_2\text{SO}_4$ + H-DMPP	2.4 ± 3.0	24.8 ± 2.7	25.6 ± 3.8	48.3 ± 6.4	60.5 ± 8.4

[0230] Further comparative tests were conducted in Dahlen soil for Compounds **18**, **20** and **23**, against H-DMPP at both 25°C and 35°C. The results of these tests are shown in Figure 5 and Table 5 below. Again, it should be noted that the considerably large error for the $\text{NO}_x\text{-N}$ measurements shown in Figures 5B and 5D is likely due to the fact that this soil was particularly rich in NO_3^- (see above).

Table 5. Ammonia nitrified (%) during a 28-day incubation at either 25°C or 35°C in Dahlen soil (pH 7.3). Emboldened values indicate nitrification rates lower than those observed in the control treatment ((NH₄)₂SO₄), correlating positively to inhibitor activity. Errors stated are standard errors of the mean, n = 3.

Test Temp (°C)	Treatment	Nitrified NH ₄ ⁺ -N (%)				
		Day 3	Day 7	Day 14	Day 21	Day 28
25	(NH ₄) ₂ SO ₄	6.4 ± 0.6	22.5 ± 1.1	45.2 ± 2.4	73.6 ± 0.9	89.7 ± 3.0
	(NH ₄) ₂ SO ₄ + 18	5.1 ± 5.3	4.4 ± 1.1	29.4 ± 2.4	32.1 ± 2.3	40.5 ± 1.5
	(NH ₄) ₂ SO ₄ + 20	1.9 ± 1.3	8.0 ± 0.6	15.2 ± 1.2	26.0 ± 2.1	31.8 ± 4.5
	(NH ₄) ₂ SO ₄ + 23	4.8 ± 2.9	15.7 ± 1.5	30.3 ± 0.8	35.9 ± 1.2	60.5 ± 1.0
	(NH ₄) ₂ SO ₄ + H-DMPP	4.9 ± 3.1	11.0 ± 3.2	17.4 ± 4.2	28.7 ± 5.2	21.8 ± 3.3
35	(NH ₄) ₂ SO ₄	17.5 ± 5.3	18.2 ± 3.6	38.4 ± 4.5	41.4 ± 12.2	65.9 ± 4.7
	(NH ₄) ₂ SO ₄ + 18	9.0 ± 4.3	4.5 ± 4.3	26.7 ± 3.9	19.8 ± 5.0	43.0 ± 4.7
	(NH ₄) ₂ SO ₄ + 20	16.6 ± 4.5	5.0 ± 4.3	17.7 ± 3.8	17.1 ± 3.6	45.6 ± 8.3
	(NH ₄) ₂ SO ₄ + 23	15.6 ± 6.4	8.8 ± 5.6	24.0 ± 6.7	41.7 ± 4.7	64.6 ± 4.9
	(NH ₄) ₂ SO ₄ + H-DMPP	15.3 ± 2.9	15.8 ± 2.5	16.0 ± 6.0	51.5 ± 5.4	38.5 ± 2.9

[0231] At 25°C, Compounds **18** and **23** performed statistically better at retaining NH₄⁺-N levels in the soil by day 28 compared to both the fertilised control and DMPP (*P* < .001). Compound **20** performed statistically better than the fertilised control (*P* < .001). However, this effectiveness was not reflected in reductions in NO_x⁻-N concentrations, where none of the treatments showed significant effectiveness on day 28 compared to the control treatment or DMPP, respectively.

[0232] At 35°C, the trends in NH₄⁺-N and NO_x⁻-N concentrations were less linear than those observed at 25°C, particularly for the NO_x⁻-N data. Compounds **18** and **20** and DMPP were the only treatments to remain highly effective at retaining NH₄⁺-N in the soil compared to the (NH₄)₂SO₄-treated control by day 28 (**18**: *P* < .01, **20**: *P* < .001). However, the large decrease in NH₄⁺-N concentration observed on day 21 for the DMPP treatment, corresponding to an essential loss of inhibitory activity (52% nitrified ammonia compared to only 41% for the control, see Table 5) was not reflected by treatments with Compounds **18** and **20**. With regards to the NO_x⁻-N data, all NIs except for DMPP showed lower NO_x⁻-N concentrations than the control on day 28, however not to a statistically significant extent.

South Johnstone Soil

[0233] The behaviour of the detected NH₄⁺-N and NO_x⁻-N concentrations differed in South Johnstone soil compared with the other soils studied. The increasing NH₄⁺-N concentrations observed over the first 14 days indicates that mineralisation of nitrogen is occurring in the soil. The test at 35°C included a water-only control treatment in addition to the (NH₄)₂SO₄-treated control, which indicated that mineralisation occurred in the soil regardless of treatment, and was stimulated by the addition of the nitrogen-based fertiliser. This 'complication' makes assessment of the impact of the tested compounds on the nitrification process more difficult, because only from day 14 onwards the NH₄⁺-N begins to show net consumption instead of net growth (from the mineralisation). The amount of ammonia lost compared to the amount detected on day 0 for selected treatments is displayed in Table 6 for tests at both 25°C and 35°C, whilst Figure 6 illustrates the measured amounts of NH₄⁺-N and NO_x⁻-N. For almost all entries, the percentages are negative, which indicates the [NH₄⁺-N] at that time point remains higher than what was detected on day 0 (due to the mineralisation process). From day 14 onwards, larger negative percentages indicate which treatments were more effective at preventing [NH₄⁺-N] losses.

[0234] At 25°C, all treatments performed significantly better than the (NH₄)₂SO₄ control treatment at both retaining NH₄⁺-N and slowing NO_x⁻-N growth on days 21 and 28 (see Figure 6). Of the treatments, Compound **19** and DMPP were the least effective (although still significantly better than the control treatment). Compared to treatment with DMPP, Compounds **3** and **18** both showed statistically higher effectiveness based on higher NH₄⁺-N and lower NO_x⁻-N concentrations on day 28 (**3**: *P* < .05 and < .01, respectively; **18**: *P* < .01 and < .001, respectively).

Table 6. Ammonia nitrified (%) during a 28-day incubation, conducted in South Johnstone soil (pH 5.0). Nitrification values calculated from ammonia levels detected in samples at each time point. All samples were treated with fertiliser $(\text{NH}_4)_2\text{SO}_4$ at a rate of 100 mg N kg^{-1} . Emboldened values indicate nitrification rates lower than those observed in the control treatment $((\text{NH}_4)_2\text{SO}_4)$, correlating positively to inhibitor activity. Values reported as means ($n = 3$); errors reported are standard errors of the mean.

Test Temp ($^{\circ}\text{C}$)	Treatment	Nitrified $\text{NH}_4^+\text{-N}$ (%)				
		Day 3	Day 7	Day 14	Day 21	Day 28
25	$(\text{NH}_4)_2\text{SO}_4$	-4.3 ± 2.3	-7.2 ± 0.7	-8.5 ± 0.8	-3.0 ± 0.8	4.2 ± 2.4
	$(\text{NH}_4)_2\text{SO}_4$ + 3	-6.3 ± 0.7	-7.9 ± 0.9	-12.7 ± 0.7	-13.9 ± 0.9	-12.8 ± 0.8
	$(\text{NH}_4)_2\text{SO}_4$ + 16	-6.3 ± 0.8	-11.5 ± 0.8	-14.4 ± 0.7	-16.8 ± 1.2	-13.2 ± 0.7
	$(\text{NH}_4)_2\text{SO}_4$ + 18	-6.2 ± 0.9	-8.5 ± 1.0	-11.0 ± 1.0	-12.1 ± 1.4	-14.9 ± 1.4
	$(\text{NH}_4)_2\text{SO}_4$ + H-DMPP	-12.5 ± 8.8	-10.3 ± 0.7	-12.5 ± 0.6	-12.0 ± 1.6	-7.5 ± 0.5
35	$(\text{NH}_4)_2\text{SO}_4$	-12.9 ± 0.6	-21.2 ± 0.9	-25.4 ± 1.1	-9.8 ± 0.72	4.1 ± 5.3
	$(\text{NH}_4)_2\text{SO}_4$ + 3	-11.8 ± 1.5	-26.2 ± 1.5	-34.8 ± 0.6	-28.3 ± 1.4	-18.6 ± 2.3
	$(\text{NH}_4)_2\text{SO}_4$ + 16	-14.7 ± 1.9	-26.3 ± 1.7	-35.7 ± 2.4	-35.7 ± 0.6	-18.4 ± 2.3
	$(\text{NH}_4)_2\text{SO}_4$ + 18	-11.7 ± 2.0	-28.7 ± 1.5	-35.8 ± 1.3	-31.7 ± 1.1	-16.9 ± 1.8
	$(\text{NH}_4)_2\text{SO}_4$ + H-DMPP	-12.3 ± 1.2	-25.3 ± 0.9	-28.1 ± 1.3	-23.0 ± 2.3	-0.90 ± 2.3

[0235] At 35°C , only the subset of Compounds **3**, **16** and **18** performed statistically better than the fertilised control at both retaining $\text{NH}_4^+\text{-N}$ and slowing $\text{NO}_x^+\text{-N}$ growth on day 28 ($P < .001$). At this temperature Compounds **3**, **16** and **18** also performed statistically better than DMPP at retaining $\text{NH}_4^+\text{-N}$ ($P < .001$ for **3** and **16**, $P < .05$ for **18**).

2.2. Leaching studies for DMP and 16

[0236] Leachability of soil nitrification inhibitors is an important consideration, due to the potential cascading health consequences that may arise if chemical inhibitors move through the soil profile and enter ground water supplies in high concentrations. It is also an important consideration for the effectiveness of the inhibitor, as high mobility in soils may lead to spatial separation between the inhibitor, NH_4^+ ions and the microorganisms involved in the nitrification process, leading to reduced field effectiveness.

[0237] Traditional soil leaching columns are both material and time intensive and could not be undertaken due to limited access to the soils of interest. Therefore, a soil thin-layer chromatography (TLC) technique was developed by modifying a method that has previously been described for the investigation of pesticide leaching behaviour (Helling, C.S., Turner, B.C, Science 1968, 162, 562-563). The advantage of the TLC technique is that data can be provided very quickly requiring only small amounts of soil and substrate.

2.2.1. General soil thin layer chromatography (TLC) plate preparation

[0238] TLC plates were prepared based on methods described in the literature (Helling, C.S., Turner, B.C, Science 1968, 162, 562-563; Mohammad, A., Jabeen, N., JPC - Journal of Planar Chromatography - Modern TLC 2003, 16, 137-143). Masking tape (3 layers, $\sim 450 \mu\text{m}$ total thickness) was used to outline three columns (4 cm W x 12 cm H) on a glass TLC plate (20 x 20 cm). A slurry of freshly ground soil in distilled H_2O ($\sim 2:3 \text{ m/v}$) was then poured onto the prepared plate and spread evenly using a glass rod. Once even, the plate was dried overnight in an oven at 35°C . Careful removal of the masking tape afforded the TLC plate ready for sample application.

2.2.2. Leaching studies of DMP and Compound 16

[0239] Samples of DMP (the active core of DMPP) or Compound **16** ($\sim 1 \text{ mg}$) dissolved in acetone ($100 \mu\text{L}$) were pipetted in a straight line across the soil, 2 cm above the base of the plate. Application band thickness was kept under 0.5 cm. After 30 mins of drying time, the TLC plate was developed inside a glass developing chamber with distilled H_2O (depth of 0.5 cm) until the solvent front reached the top of the soil ($\sim 1 \text{ hr}$). If the solvent front failed to move through the three adjacent soil channels at the same rate, the plate was removed once the solvent reached the top of one channel, with any dry soil in the remaining channels carefully scraped away to mark the height of the solvent front. The plate was then allowed to air dry overnight.

[0240] Once dry, the plate was divided into six horizontal bands corresponding to R_f values of: (1) <0.05 (baseline), (2) 0.05 to 0.25, (3) 0.25 to 0.45, (4) 0.45 to 0.65, (5) 0.65 to 0.85, and (6) 0.85 to 1. In sequence, soil in each band

was carefully scraped off the glass backing and collected in vials. Special care was taken to avoid cross-contamination between soil of different bands, and the separate channels.

2.2.3. Extraction and analysis of DMP and Compound 16

[0241] Individual soil bands collected from the TLC plate were extracted as follows:

1. Addition of an aqueous solution of $\text{CaCl}_2/\text{MgSO}_4$ (0.01M and 0.45M respectively, 2 mL).
2. Sonication for 5 minutes then manual shaking for 30 seconds.
3. Addition of methyl-tert-butyl ether (MTBE, 2 mL) then manual shaking for 30 seconds.
4. Sonication for 10 minutes then manual shaking for 30 seconds.
5. Rested until soil began to settle, then frozen overnight at -20°C .
6. After partial defrosting, the ethereal extract was filtered through nylon syringe filters (FilterBio®, 13 mm diameter, 0.45 μm pore size).

[0242] The filtered ethereal extracts (450 μL) were spiked with 50 μL of a standard solution of either cyclododecanone in MTBE (1.97 mg/mL, for DMP-treated samples) or cyclooctanone in MTBE (6.37 mg/mL, for 16-treated samples). Samples were then directly analysed by GC-MS (method: 70°C for 5 mins, then ramp $10^\circ\text{C}/\text{min}$ to 250°C , hold at 250°C for 17 mins [total run time = 40 minutes]) to analyse the presence or absence of the inhibitor in each soil band.

2.2.4. Leached inhibitor calculations

[0243] GC-MS peak areas calculated for the standards cyclooctanone ($R_t = 9.1$ mins) and cyclododecanone ($R_t = 15.8$ mins) were compared to those calculated for Compound 16 ($R_t = 13.9$ mins) and DMP ($R_t = 7.6$ mins) respectively, for each soil sample extract where inhibitor was detected as follows:

$$\text{Ratio}_{\text{Area}} = \frac{\text{Peak Area}_{\text{inhibitor}}}{\text{Peak Area}_{\text{standard}}}$$

then for samples from a single TLC channel;

$$\% \text{ Detected inhibitor (per } R_f \text{ band)} = \frac{\text{Ratio}_{\text{Area}} (\text{specific } R_f \text{ band})}{\text{Sum of Ratio}_{\text{Area}} \text{ of all } R_f \text{ bands}} \times 100$$

[0244] As each treatment was run in triplicate, mean values are reported for detected inhibitor percentages for each R_f band, with errors presented as the standard deviation.

Table 7. Results from soil Thin-Layer Chromatography (TLC) to assess the leaching potential of inhibitors DMP and Compound 16 in two soils.

R_f	Detected Inhibitor (%) ^a			
	16 ^b		DMP ^c	
	Dahlen	South Johnstone	Dahlen	South Johnstone
<0.05	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>
0.05 - 0.25	3 $\pm$ 4	1 $\pm$ 1	<i>n.d.</i>	<i>n.d.</i>
0.25 - 0.45	58 $\pm$ 14	5 $\pm$ 6	<i>n.d.</i>	2 $\pm$ 2
0.45 - 0.65	38 $\pm$ 16	34 $\pm$ 5	0.1 $\pm$ 0.2	56 $\pm$ 12
0.65 - 0.85	1 $\pm$ 1	49 $\pm$ 9	72 $\pm$ 16	42 $\pm$ 13
0.85 - 1	<i>n.d.</i>	11 $\pm$ 3	28 $\pm$ 16	<i>n.d.</i>

^a Means of three replicates, error presented is the standard deviation. ^b Values were calculated compared to internal standard cyclooctanone. ^c Values were calculated compared to internal standard cyclododecanone. Not detected = *n.d.*

2.2.5. Leaching studies of DCD

[0245] Samples of the DCD (~1 mg) dissolved in methanol (300 μ L) were pipetted in a straight line across the soil, 2 cm above the base of the plate. Application band thickness was kept under 0.5 cm. After 30 mins of drying time, the TLC plate was developed inside a glass developing chamber in distilled H₂O (depth of 0.5 cm) until the solvent front reached the top of the soil (~1 hr). If the solvent front failed to move through the three adjacent soil channels at the same rate, the plate was removed once the solvent reached the top of the soil in one channel, with any dry soil in the remaining channels carefully scraped away to mark the height of the solvent front. The plate was then allowed to air dry overnight.

[0246] Once dry, the plate was divided into six horizontal bands corresponding to R_f values of: (1) <0.05 (baseline), (2) 0.05 to 0.25, (3) 0.25 to 0.45, (4) 0.45 to 0.65, (5) 0.65 to 0.85, and (6) 0.85 to 1. In sequence, soil in each band was carefully scraped off the glass backing and collected in vials. Special care was taken to avoid cross-contamination between soil of different bands, and the separate channels.

2.2.6. Extraction and analysis of DCD

[0247] Individual soil bands collected from the TLC plate were extracted as follows:

1. Addition of methanol (2 mL).
2. Manual shaking for 30 seconds following sonication for 15 minutes.
3. Once soil began to settle, the methanolic extract was filtered through nylon syringe filters (FilterBio®, 13 mm diameter, 0.45 μ m pore size).
4. 300 μ L of the methanolic extracts were evaporated under nitrogen flow, and then the residues were taken up in ultrapure acetonitrile (1 mL).
5. Acetonitrile solutions were filtered through PVDF syringe filters (Millex®, 33 mm diameter, 0.22 μ m pore size).

[0248] The filtered acetonitrile extracts (10 μ L injection) were then directly analysed by HPLC (1260 Infinity II Preparative LC system with a C18 column, Agilent) to detect the presence or absence of DCD in each soil band at 214 nm. The HPLC method used was as follows:

- Solvent A: 0.1% formic acid in H₂O
- Solvent B: 0.1% formic acid in acetonitrile
- Ramp from 100% A to 100% B over ten mins. Hold at 100% B for two mins then return to 100% A in 10 secs, for a 2-minute wash at 100% A. Total sample run time = 15 mins.

2.2.7. Results

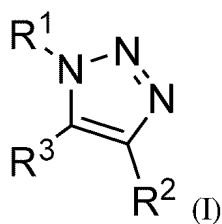
[0249] The retention factor (R_f) is used to measure the movement of compounds through the soil using the TLC method, with a high R_f -value close to 1 indicating high mobility through the soil. In the neutral Dahlen soil, Compound **16** showed reduced mobility compared to DMP, with the majority of the triazole detected in the R_f range 0.25-0.45, versus 0.65-0.85 for DMP (see Figure 7). For the acidic South Johnstone soil, DMP was found to leach in a narrower band and to a lesser extent than Compound **16**. This may be due to protonation of DMP in lower pH environments. The resulting charged molecule may be adsorbed on the soil particles, therefore reducing leaching. However, since DMP is not the target of this investigation, the underlying process was not explored.

[0250] Dicyandiamide (DCD) is a widely used nitrification inhibitor, which, due to its high water solubility, has known leaching concerns. Preliminary results from TLC leaching studies of DCD in both the Dahlen and South Johnstone soils show the largest DCD accumulation in the R_f range 0.65-1. This result contradicts the correlation between protonation ease and reduced mobility, as DCD has multiple protonation sites and would therefore be expected to leach less.

[0251] The results from these leaching studies indicate that in neutral soils Compound **16**, and by extension other similar small lipophilic triazoles, have lower leachability than DMP and DCD. Acidic soils again seem to be potentially more problematic, however Compound **16** does still appear to show lower-to-similar leaching tendencies to DCD.

Claims

1. A method for reducing nitrification in soil comprising treating the soil with a compound of Formula (I):



wherein

R¹ and R² are independently selected from optionally substituted -C₁-C₁₀alkyl, -C₂-C₁₀alkenyl, -C₂-C₁₀alkynyl, -C₁-C₁₀alkylC(O)OR⁴, -C₂-C₁₀alkenylC(O)OR⁴, -C₂-C₁₀alkynylC(O)OR⁴, -C₁-C₁₀alkylOC(O)R⁴, -C₂-C₁₀alkenylOC(O)R⁴, -C₂-C₁₀alkynylOC(O)R⁴, -C₁-C₁₀alkylOC(O)OR⁴, -C₂-C₁₀alkenylOC(O)OR⁴, -C₂-C₁₀alkynylOC(O)OR⁴, -C₁-C₁₀alkylC(O)N(R⁵R⁶), -C₂-C₁₀alkenylC(O)N(R⁵R⁶), -C₂-C₁₀alkynylC(O)N(R⁵R⁶), -C₁-C₁₀alkylNR⁵C(O)R⁶, -C₂-C₁₀alkenylNR⁵C(O)R⁶ and -C₂-C₁₀alkynylNR⁵C(O)R⁶;

R³ is H or is selected from optionally substituted -C₁-C₁₀alkyl, -C₂-C₁₀alkenyl, -C₂-C₁₀alkynyl, -C₁-C₁₀alkylC(O)OR⁴, -C₂-C₁₀alkenylC(O)OR⁴, -C₂-C₁₀alkynylC(O)OR⁴, -C₁-C₁₀alkylOC(O)R⁴, -C₂-C₁₀alkenylOC(O)R⁴, -C₂-C₁₀alkynylOC(O)R⁴, -C₁-C₁₀alkylOC(O)OR⁴, -C₂-C₁₀alkenylOC(O)OR⁴, -C₂-C₁₀alkynylOC(O)OR⁴, -C₁-C₁₀alkylC(O)N(R⁵R⁶), -C₂-C₁₀alkenylC(O)N(R⁵R⁶), -C₂-C₁₀alkynylC(O)N(R⁵R⁶), -C₁-C₁₀alkylNR⁵C(O)R⁶, -C₂-C₁₀alkenylNR⁵C(O)R⁶ and -C₂-C₁₀alkynylNR⁵C(O)R⁶;

R⁴ is selected from -C₁-C₄alkyl, -C₂-C₄alkenyl and -C₂-C₄alkynyl; and

R⁵ and R⁶ are independently selected from H, -C₁-C₄alkyl, -C₂-C₄alkenyl and -C₂-C₄alkynyl; or agriculturally acceptable salts thereof.

2. A method according to claim 1, wherein for the compound of Formula (I):

R¹ and R² are independently selected from -C₁-C₁₀alkyl, -C₂-C₁₀alkenyl, -C₂-C₁₀alkynyl, -C₁-C₁₀alkylC(O)OR⁴, -C₂-C₁₀alkenylC(O)OR⁴, -C₂-C₁₀alkynylC(O)OR⁴, -C₁-C₁₀alkylOC(O)R⁴, -C₂-C₁₀alkenylOC(O)R⁴, -C₂-C₁₀alkynylOC(O)R⁴, -C₁-C₁₀alkylOC(O)OR⁴, -C₂-C₁₀alkenylOC(O)OR⁴, -C₂-C₁₀alkynylOC(O)OR⁴, -C₁-C₁₀alkylC(O)N(R⁵R⁶), -C₂-C₁₀alkenylC(O)N(R⁵R⁶), -C₂-C₁₀alkynylC(O)N(R⁵R⁶), -C₁-C₁₀alkylNR⁵C(O)R⁶, -C₂-C₁₀alkenylNR⁵C(O)R⁶ and -C₂-C₁₀alkynylNR⁵C(O)R⁶ optionally substituted with one or more amino, hydroxy, C₁-C₄alkoxy- or a 3-10-membered monocyclic or fused bicyclic heteroaryl comprising one or more heteroatoms selected from N, O and S, wherein said heteroaryl is optionally substituted with one or more C₁-C₁₀alkyl, oxo, hydroxy, C₁-C₄alkoxy- or amino;

R³ is H or is selected from -C₁-C₁₀alkyl, -C₂-C₁₀alkenyl, -C₂-C₁₀alkynyl, -C₁-C₁₀alkylC(O)OR⁴, -C₂-C₁₀alkenylC(O)OR⁴, -C₂-C₁₀alkynylC(O)OR⁴, -C₁-C₁₀alkylOC(O)R⁴, -C₂-C₁₀alkenylOC(O)R⁴, -C₂-C₁₀alkynylOC(O)R⁴, -C₁-C₁₀alkylOC(O)OR⁴, -C₂-C₁₀alkenylOC(O)OR⁴, -C₂-C₁₀alkynylOC(O)OR⁴, -C₁-C₁₀alkylC(O)N(R⁵R⁶), -C₂-C₁₀alkenylC(O)N(R⁵R⁶), -C₂-C₁₀alkynylC(O)N(R⁵R⁶), -C₁-C₁₀alkylNR⁵C(O)R⁶, -C₂-C₁₀alkenylNR⁵C(O)R⁶ and -C₂-C₁₀alkynylNR⁵C(O)R⁶ optionally substituted with one or more amino, hydroxy, C₁-C₄alkoxy- or a 3-10-membered monocyclic or fused bicyclic heteroaryl comprising one or more heteroatoms selected from N, O and S, wherein said heteroaryl is optionally substituted with one or more C₁-C₁₀alkyl, oxo, hydroxy, C₁-C₄alkoxy- or amino;

R⁴ is selected from -C₁-C₄alkyl, -C₂-C₄alkenyl and -C₂-C₄alkynyl; and

R⁵ and R⁶ are independently selected from H, -C₁-C₄alkyl, -C₂-C₄alkenyl and -C₂-C₄alkynyl.

3. A method according to claim 1 or 2, wherein the soil is co-treated with a urease inhibitor.

4. A method according to any one of claims 1 to 3, wherein the soil is co-treated with a fertiliser.

5. The use of a composition for reducing nitrification, the composition comprising a compound of Formula (I) as defined in claim 1 or 2.

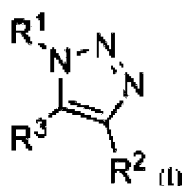
6. The use of a composition for reducing nitrification according to claim 5, the composition comprising at least one agriculturally acceptable adjuvant or diluent.

7. The use of a composition for reducing nitrification according to either claim 5 or claim 6, wherein the composition further comprises a urease inhibitor.

8. A fertiliser comprising a urea- or ammonium-based fertiliser and a compound of Formula (I) as defined in claim 1 or 2.
9. A fertiliser according to claim 8 further comprising a urease inhibitor.
10. A fertiliser according to claim 8 or 9, wherein the urea- or ammonium-based fertiliser is in the form of a granule and the compound of Formula (I) and optionally the urease inhibitor are coated on the granule.

Patentansprüche

1. Verfahren zur Verringerung der Nitrifikation im Boden, umfassend die Behandlung des Bodens mit einer Verbindung der Formel (I):



wobei

R¹ und R² unabhängig voneinander ausgewählt sind aus gegebenenfalls substituierten -C₁-C₁₀-Alkyl, -C₂-C₁₀-Alkenyl, -C₂-C₁₀-Alkynyl, -C₁-C₁₀-AlkylC(O)OR⁴, -C₂-C₁₀-Alkenyl-C(O)OR⁴, -C₂-C₁₀-Alkynyl-C(O)OR⁴, -C₁-C₁₀-Alkyl-OC(O)R⁴, -C₂-C₁₀-Alkenyl-OC(O)R⁴, -C₂-C₁₀-Alkynyl-OC(O)R⁴, -C₁-C₁₀-Alkyl-OC(O)OR⁴, -C₂-C₁₀-Alkenyl-OC(O)OR⁴, -C₂-C₁₀-Alkynyl-OC(O)OR⁴, -C₁-C₁₀-Alkyl-C(O)N(R⁵R⁶), -C₂-C₁₀-Alkenyl-C(O)N(R⁵R⁶), -C₂-C₁₀-Alkynyl-C(O)N(R⁵R⁶), -C₁-C₁₀-Alkyl-NR⁵C(O)R⁶, -C₂-C₁₀-Alkenyl-NR⁵C(O)R⁶ und -C₂-C₁₀-Alkynyl-NR⁵C(O)R⁶;

R³ ist H oder ausgewählt aus gegebenenfalls substituierten -C₁-C₁₀-Alkyl, -C₂-C₁₀-Alkenyl, -C₂-C₁₀-Alkynyl, -C₁-C₁₀-AlkylC(O)OR⁴, -C₂-C₁₀-Alkenyl-C(O)OR⁴, -C₂-C₁₀-Alkynyl-C(O)OR⁴, -C₁-C₁₀-Alkyl-OC(O)R⁴, -C₂-C₁₀-Alkenyl-OC(O)R⁴, -C₂-C₁₀-Alkynyl-OC(O)R⁴, -C₁-C₁₀-Alkyl-OC(O)OR⁴, -C₂-C₁₀-Alkenyl-OC(O)OR⁴, -C₂-C₁₀-Alkynyl-OC(O)OR⁴, -C₁-C₁₀-Alkyl-C(O)N(R⁵R⁶), -C₂-C₁₀-Alkenyl-C(O)N(R⁵R⁶), -C₂-C₁₀-Alkynyl-C(O)N(R⁵R⁶), -C₁-C₁₀-Alkyl-NR⁵C(O)R⁶, -C₂-C₁₀-Alkenyl-NR⁵C(O)R⁶ und -C₂-C₁₀-Alkynyl-NR⁵C(O)R⁶;

R⁴ ist ausgewählt aus -C₁-C₄-Alkyl, -C₂-C₄-Alkenyl und -C₂-C₄-Alkynyl; und

R⁵ und R⁶ sind unabhängig voneinander ausgewählt aus H, -C₁-C₄-Alkyl, -C₂-C₄-Alkenyl und -C₂-C₄-Alkynyl; oder landwirtschaftlich verträgliche Salze davon.

2. Verfahren nach Anspruch 1, wobei für die Verbindung der Formel (I):

R¹ und R² sind unabhängig voneinander ausgewählt aus -C₁-C₁₀-Alkyl, -C₂-C₁₀-Alkenyl, -C₂-C₁₀-Alkynyl, -C₁-C₁₀-AlkylC(O)OR⁴, -C₂-C₁₀-Alkenyl-C(O)OR⁴, -C₂-C₁₀-Alkynyl-C(O)OR⁴, -C₁-C₁₀-Alkyl-OC(O)R⁴, -C₂-C₁₀-Alkenyl-OC(O)R⁴, -C₂-C₁₀-Alkynyl-OC(O)R⁴, -C₁-C₁₀-Alkyl-OC(O)OR⁴, -C₂-C₁₀-Alkenyl-OC(O)OR⁴, -C₂-C₁₀-Alkynyl-OC(O)OR⁴, -C₁-C₁₀-Alkyl-C(O)N(R⁵R⁶), -C₂-C₁₀-Alkenyl-C(O)N(R⁵R⁶), -C₂-C₁₀-Alkynyl-C(O)N(R⁵R⁶), -C₁-C₁₀-Alkyl-NR⁵C(O)R⁶, -C₂-C₁₀-Alkenyl-NR⁵C(O)R⁶ und -C₂-C₁₀-Alkynyl-NR⁵C(O)R⁶, die gegebenenfalls mit einer oder mehreren Amino-, Hydroxy-, C₁-C₄-Alkoxy-Gruppen oder einem 3- bis 10-gliedrigen monocyclischen oder kondensierten bicyclischen Heteroaryl substituiert sind, der ein oder mehrere Heteroatome umfasst, ausgewählt aus N, O und S, wobei das Heteroaryl gegebenenfalls mit einem oder mehreren C₁-C₁₀-Alkyl, Oxo, Hydroxy, C₁-C₄-Alkoxy- oder Aminogruppen substituiert ist;

R³ ist H oder ausgewählt aus -C₁-C₁₀-Alkyl, -C₂-C₁₀-Alkenyl, -C₂-C₁₀-Alkynyl, -C₁-C₁₀-AlkylC(O)OR⁴, -C₂-C₁₀-Alkenyl-C(O)OR⁴, -C₂-C₁₀-Alkynyl-C(O)OR⁴, -C₁-C₁₀-Alkyl-OC(O)R⁴, -C₂-C₁₀-Alkenyl-OC(O)R⁴, -C₂-C₁₀-Alkynyl-OC(O)R⁴, -C₁-C₁₀-Alkyl-OC(O)OR⁴, -C₂-C₁₀-Alkenyl-OC(O)OR⁴, -C₂-C₁₀-Alkynyl-OC(O)OR⁴, -C₁-C₁₀-Alkyl-C(O)N(R⁵R⁶), -C₂-C₁₀-Alkenyl-C(O)N(R⁵R⁶), -C₂-C₁₀-Alkynyl-C(O)N(R⁵R⁶), -C₁-C₁₀-Alkyl-NR⁵C(O)R⁶, -C₂-C₁₀-Alkenyl-NR⁵C(O)R⁶ und -C₂-C₁₀-Alkynyl-NR⁵C(O)R⁶, die gegebenenfalls mit einer oder mehreren Amino-, Hydroxy-, C₁-C₄-Alkoxy-Gruppen oder einem 3- bis 10-gliedrigen monocyclischen oder kondensierten bicyclischen Heteroaryl substituiert sind, der ein oder mehrere Heteroatome umfasst, ausgewählt aus N, O und S, wobei das Heteroaryl gegebenenfalls mit einem oder mehreren C₁-C₁₀-Alkyl, Oxo, Hydroxy, C₁-C₄-Alkoxy oder Aminogruppen substituiert ist;

R⁴ est ausgewählt aus -C₁-C₄-Alkyl, -C₂-C₄-Alkenyl und -C₂-C₄-Alkynyl; und

R⁵ und R⁶ sind unabhängig voneinander ausgewählt aus H, -C₁-C₄-Alkyl, -C₂-C₄-Alkenyl und -C₂-C₄-Alkynyl.

3. Verfahren nach Anspruch 1 oder 2, wobei der Boden gleichzeitig mit einem Ureasehemmer behandelt wird.

4. Verfahren nach einem der Ansprüche 1 bis 3, wobei der Boden gleichzeitig mit einem Dünger behandelt wird.

5. Verwendung einer Zusammensetzung zur Verringerung der Nitrifikation, wobei die Zusammensetzung eine Verbindung der Formel (I) wie in Anspruch 1 oder 2 definiert, umfasst.

6. Verwendung einer Zusammensetzung zur Verringerung der Nitrifikation gemäß Anspruch 5, wobei die Zusammensetzung mindestens ein landwirtschaftlich verträgliches Hilfsmittel oder Verdünnungsmittel umfasst.

7. Verwendung einer Zusammensetzung zur Verringerung der Nitrifikation gemäß Anspruch 5 oder Anspruch 6, wobei die Zusammensetzung ferner einen Ureasehemmer enthält.

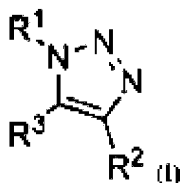
8. Düngemittel, das ein Düngemittel auf Harnstoff- oder Ammoniumbasis und eine Verbindung der Formel (I), wie in Anspruch 1 oder 2 definiert, enthält.

9. Düngemittel gemäß Anspruch 8, das ferner einen Ureasehemmer enthält.

10. Düngemittel gemäß Anspruch 8 oder 9, wobei das Düngemittel auf Harnstoff- oder Ammoniumbasis in Form eines Granulats vorliegt und die Verbindung der Formel (I) sowie gegebenenfalls der Ureasehemmer auf das Granulat aufgebracht sind.

Revendications

1. Procédé pour réduire la nitrification dans le sol, comprenant le traitement du sol avec un composé de formule (I) :



dans lequel

R¹ et R² sont choisis indépendamment parmi les groupes suivants éventuellement substitués :

-alkyle en C₁ à C₁₀, -alcényle en C₂ à C₁₀, -alcynyle en C₂ à C₁₀, -alkyl en C₁ à C₁₀C(O)OR⁴, -alcényle en C₂ à C₁₀C(O)OR⁴, -alcynyle en C₂ à C₁₀C(O)OR⁴, -alkyle en C₁ à C₁₀OC(O)R⁴, -alcényle en C₂ à C₁₀OC(O)R⁴, -alcynyle en C₂ à C₁₀OC(O)R⁴, -alkyle en C₁ à C₁₀OC(O)OR⁴, -alcényle en C₂ à C₁₀OC(O)OR⁴, -alcynyle en C₂ à C₁₀OC(O)OR⁴, -alkyle en C₁ à C₁₀C(O)N(R⁵R⁶), -alcényle en C₂ à C₁₀C(O)N(R⁵R⁶), -alcynyle en C₂ à C₁₀C(O)N(R⁵R⁶), -alkyle en C₁ à C₁₀-NR⁵-C(O)R⁶, -alcényle en C₂ à C₁₀-NR⁵-C(O)R⁶ et -alcynyle en C₂ à C₁₀-NR⁵-C(O)R⁶ ;

R³ est H ou est choisi parmi les groupes suivants éventuellement substitués : -alkyle en C₁ à C₁₀,

-alcényle en C₂ à C₁₀, -alcynyle en C₂ à C₁₀, -alkyle en C₁ à C₁₀C(O)OR⁴, -alcényle en C₂ à C₁₀C(O)OR⁴, -alcynyle en C₂ à C₁₀C(O)OR⁴, -alkyle en C₁ à C₁₀COC(O)R⁴, -alcényle en C₂ à C₁₀OC(O)R⁴, -alcynyle en C₂ à C₁₀OC(O)R⁴, -alkyle en C₁ à C₁₀OC(O)OR⁴, -alcényle en C₂ à C₁₀OC(O)OR⁴, -alcynyle en C₂ à C₁₀OC(O)OR⁴, -alkyle en C₁ à C₁₀C(O)N(R⁵R⁶), -alcényle en C₂ à C₁₀C(O)N(R⁵R⁶), -alcynyle en C₂ à C₁₀C(O)N(R⁵R⁶), -alkyle en C₁ à C₁₀NR⁵C(O)R⁶, -alcényle en C₂ à C₁₀NR⁵C(O)R⁶ et -alcynyle en C₂ à C₁₀NR⁵C(O)R⁶ ;

R⁴ est choisi parmi les groupes -alkyle en C₁ à C₄, -alcényle en C₂ à C₄ et -alcynyle en C₂ à C₄ ; et

R⁵ et R⁶ sont choisis indépendamment parmi les groupes H, -alkyle en C₁ à C₄, -alcényle en C₂ à C₄ et -alcynyle en C₂ à C₄ ;

ou leurs sels acceptables en agriculture.

2. Procédé selon la revendication 1, dans lequel, pour le composé de formule (I) :

R¹ et R² sont choisis indépendamment parmi les groupes -alkyle en C₁ à C₁₀, -alcényle en C₂ à C₁₀, -alcynyle en C₂ à C₁₀, -alkyle en C₁ à C₁₀C(O)OR⁴, -alcényle en C₂ à C₁₀C(O)OR⁴, -alcynyle en C₂ à C₁₀C(O)OR⁴, -alkyle en C₁ à C₁₀OC(O)R⁴, -alcényle en C₂ à C₁₀OC(O)R⁴, -alcynyle en C₂ à C₁₀-OC(O)R⁴, -alkyle en C₁ à C₁₀-OC(O)OR⁴, -alcényle en C₂ à C₁₀OC(O)OR⁴, -alcynyle en C₂ à C₁₀-OC(O)OR⁴, -alkyle en C₁-C₁₀-(O)N(R⁵R⁶), -alcényle en C₂ à C₁₀C(O)N(R⁵R⁶), -alcynyle en C₂ à C₁₀-C(O)N(R⁵R⁶), -alkyle en C₁-C₁₀-NR⁵-C(O)R⁶, -alcényle en C₂ à C₁₀-NR⁵C(O)R⁶ et -alcynyle en C₂ à C₁₀NR⁵C(O)R⁶ éventuellement substitués par un ou plusieurs groupes amino, hydroxy, alcoxy en C₁-C₄ ou par un hétéroaryle monocyclique ou bicyclique condensé de 3 à 10 chaînons comprenant un ou plusieurs hétéroatomes choisis parmi N, O et S, ledit hétéroaryle étant éventuellement substitué par un ou plusieurs groupes -alkyle en C₁ à C₁₀, oxo, hydroxy, alcoxy en C₁ à C₄ ou amino ; R³ est H ou est choisi parmi les groupes -alkyle en C₁ à C₁₀, -alcényle en C₂ à C₁₀, -alcynyle en C₂ à C₁₀, -alkyle en C₁ à C₁₀C(O)OR⁴, -alcényle en C₂-C₁₀C(O)OR⁴, -alcynyle en C₂-C₁₀C(O)OR⁴, -alkyle en C₁-C₁₀OC(O)R⁴, -alcényle en C₂-C₁₀OC(O)R⁴, -alcynyle en C₂-C₁₀OC(O)R⁴, -alkyle en C₂-C₁₀OC(O)OR⁴, -alcényle en C₂-C₁₀OC(O)OR⁴, -alcynyle en C₂-C₁₀OC(O)OR⁴, -alkyle en C₁-C₁₀OC(O)N(R⁵R⁶), -alcényle en C₂-C₁₀OC(O)N(R⁵R⁶), -alcynyle en C₂-C₁₀OC(O)N(R⁵R⁶), -alkyle en C₁-C₁₀NR⁵C(O)R⁶, -alcényle en C₂ à C₁₀NR⁵C(O)R⁶ et -alcynyle en C₂ à C₁₀NR⁵C(O)R⁶, éventuellement substitués par un ou plusieurs groupes amino, hydroxy, alcoxy en C₁ à C₄ ou par un hétéroaryle monocyclique ou bicyclique condensé de 3 à 10 chaînons comprenant un ou plusieurs hétéroatomes choisis parmi N, O et S, ledit hétéroaryle étant éventuellement substitué par un ou plusieurs groupes -alkyle en C₁ à C₁₀, oxo, hydroxy, alcoxy en C₁ à C₄ ou amino ; R⁴ est choisi parmi les groupes -alkyle en C₁ à C₄, -alcényle en C₂ à C₄ et -alcynyle en C₂ à C₄ ; et R⁵ et R⁶ sont choisis indépendamment parmi H, -alkyle en C₁ à C₄, -alcényle en C₂ à C₄ et -alcynyle en C₂ à C₄.

3. Procédé selon la revendication 1 ou 2, dans lequel le sol est traité conjointement avec un inhibiteur d'uréase.

4. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel le sol est traité conjointement avec un engrais.

5. Utilisation d'une composition destinée à réduire la nitrification, ladite composition comprenant un composé de formule (I) tel que défini dans la revendication 1 ou 2.

6. Utilisation d'une composition destinée à réduire la nitrification selon la revendication 5, ladite composition comprenant au moins un adjuvant ou un diluant acceptable en agriculture.

7. Utilisation d'une composition destinée à réduire la nitrification selon la revendication 5 ou la revendication 6, dans laquelle la composition comprend en outre un inhibiteur d'uréase.

8. Engrais comprenant un engrais à base d'urée ou d'ammonium et un composé de formule (I) tel que défini dans la revendication 1 ou 2.

9. Engrais selon la revendication 8, comprenant en outre un inhibiteur d'uréase.

10. Engrais selon la revendication 8 ou 9, dans lequel l'engrais à base d'urée ou d'ammonium se présente sous la forme d'un granulé et le composé de formule (I) et, éventuellement, l'inhibiteur d'uréase sont enrobés sur le granulé.

Figure 1

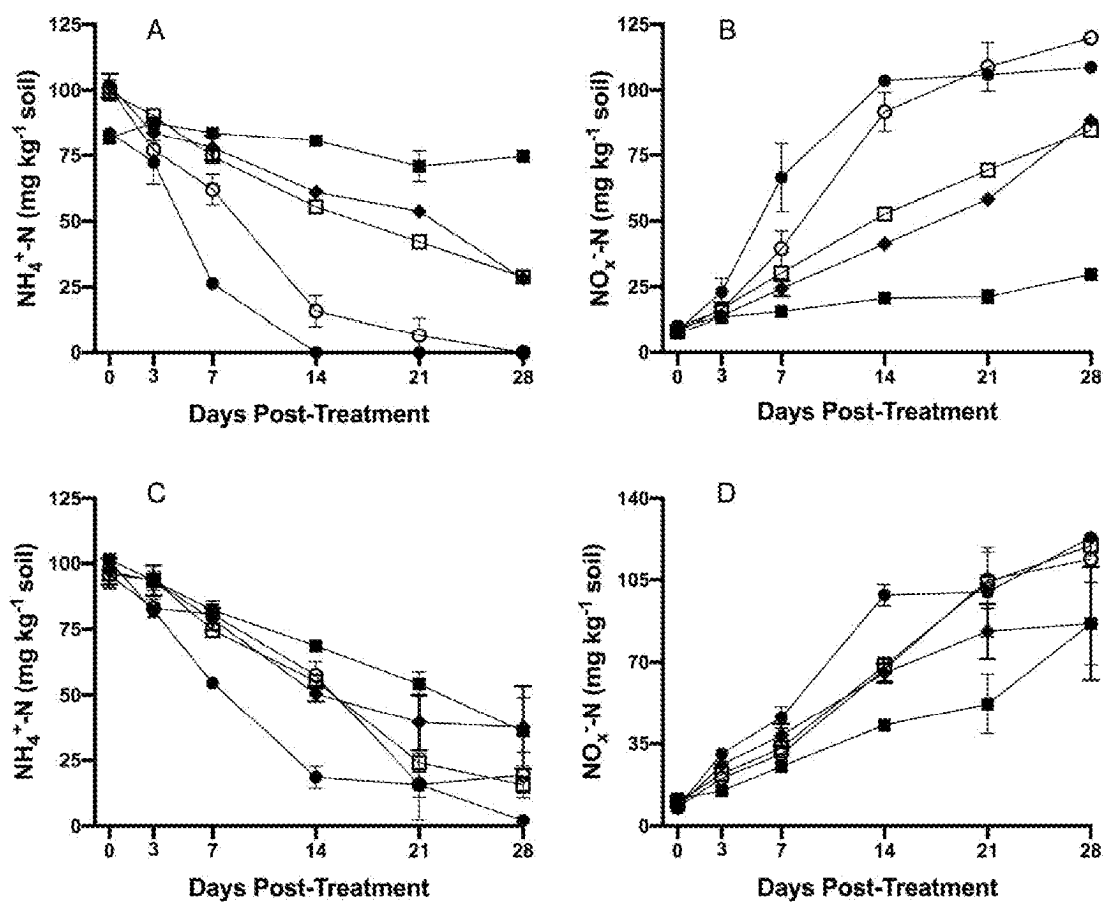


Figure 2

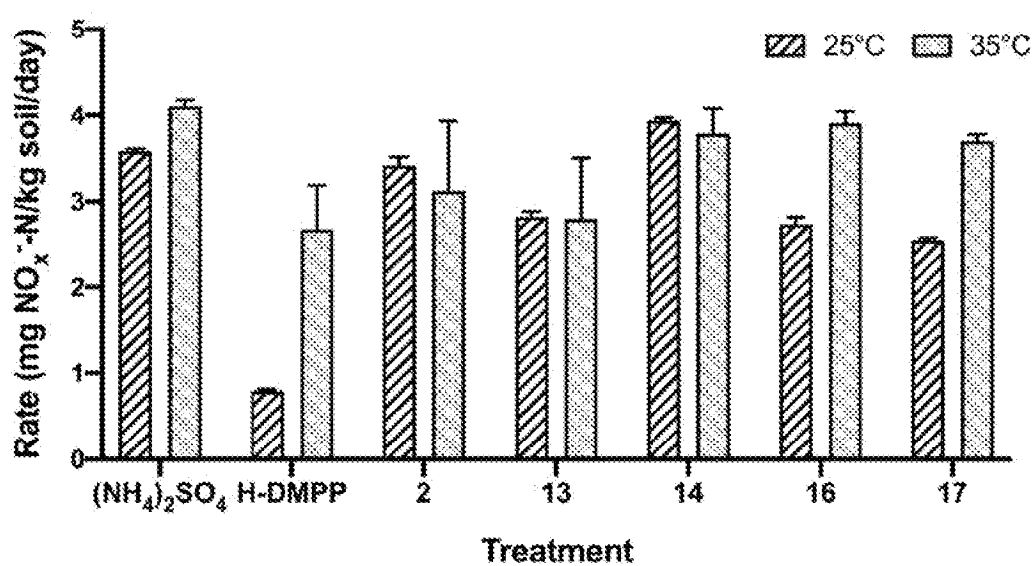


Figure 3

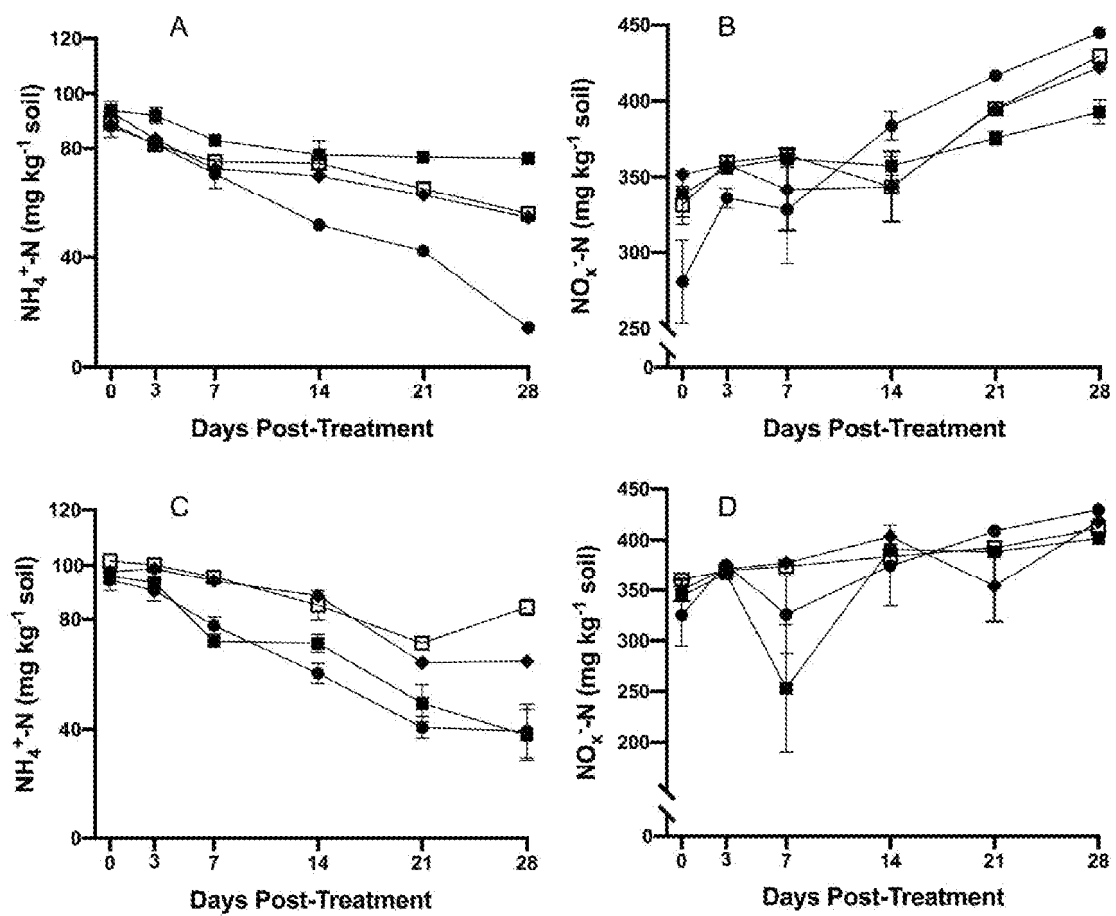


Figure 4

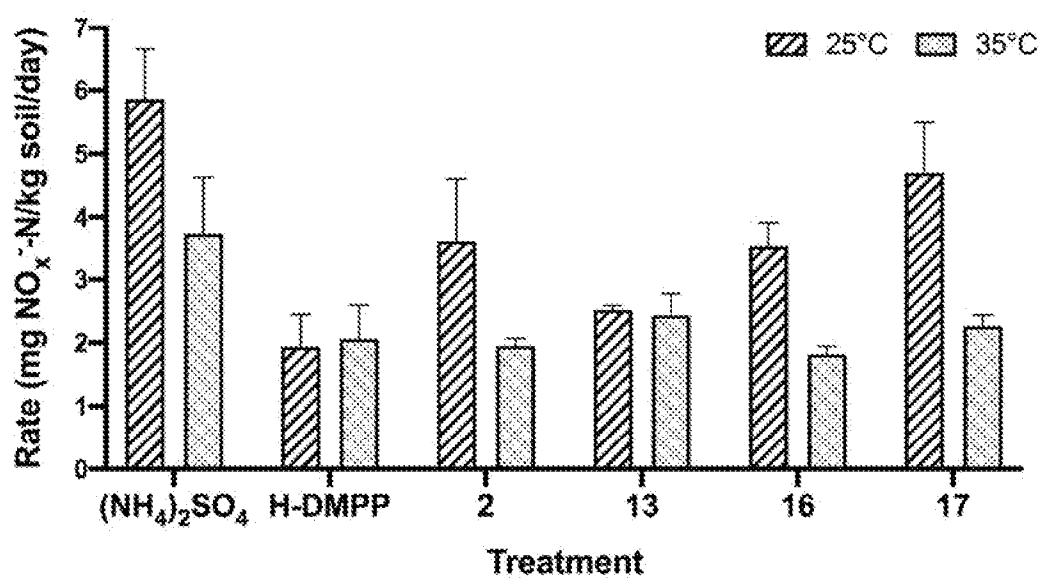


Figure 5

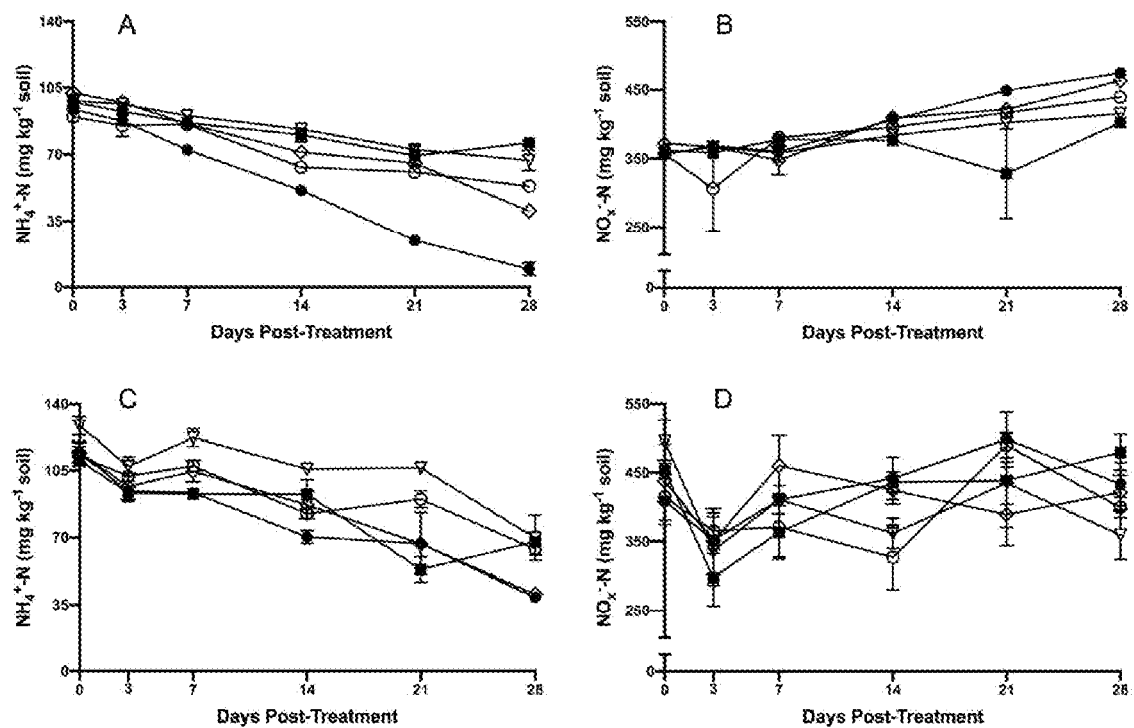


Figure 6

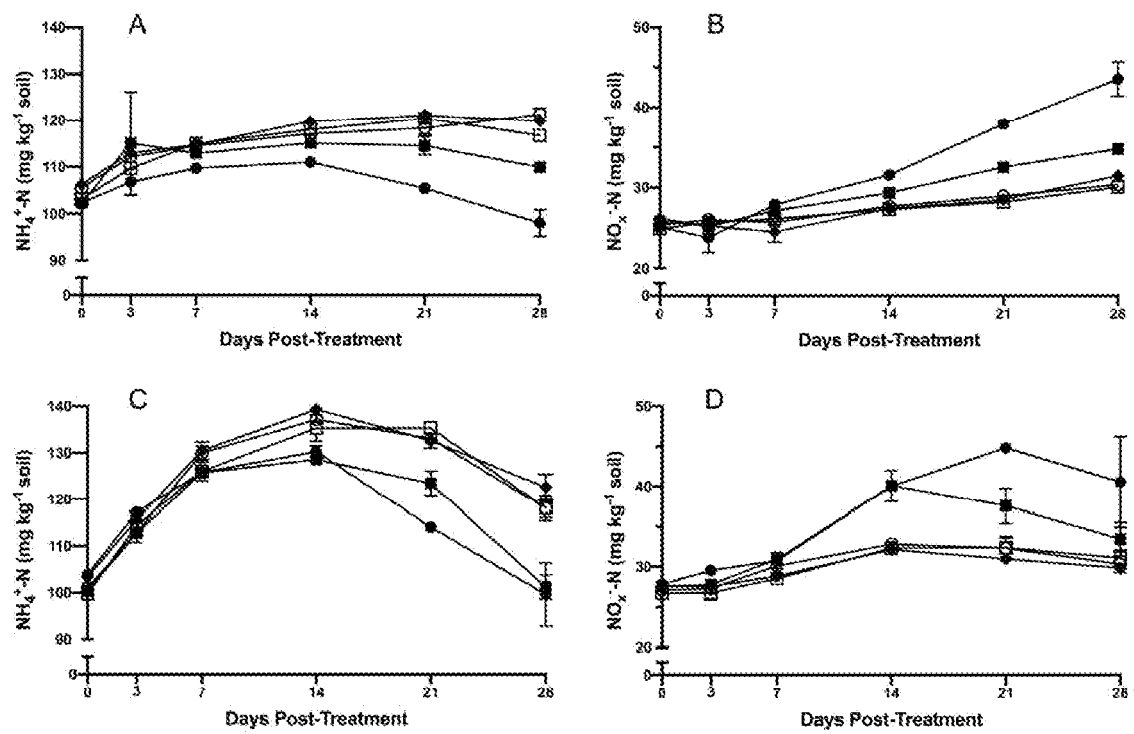
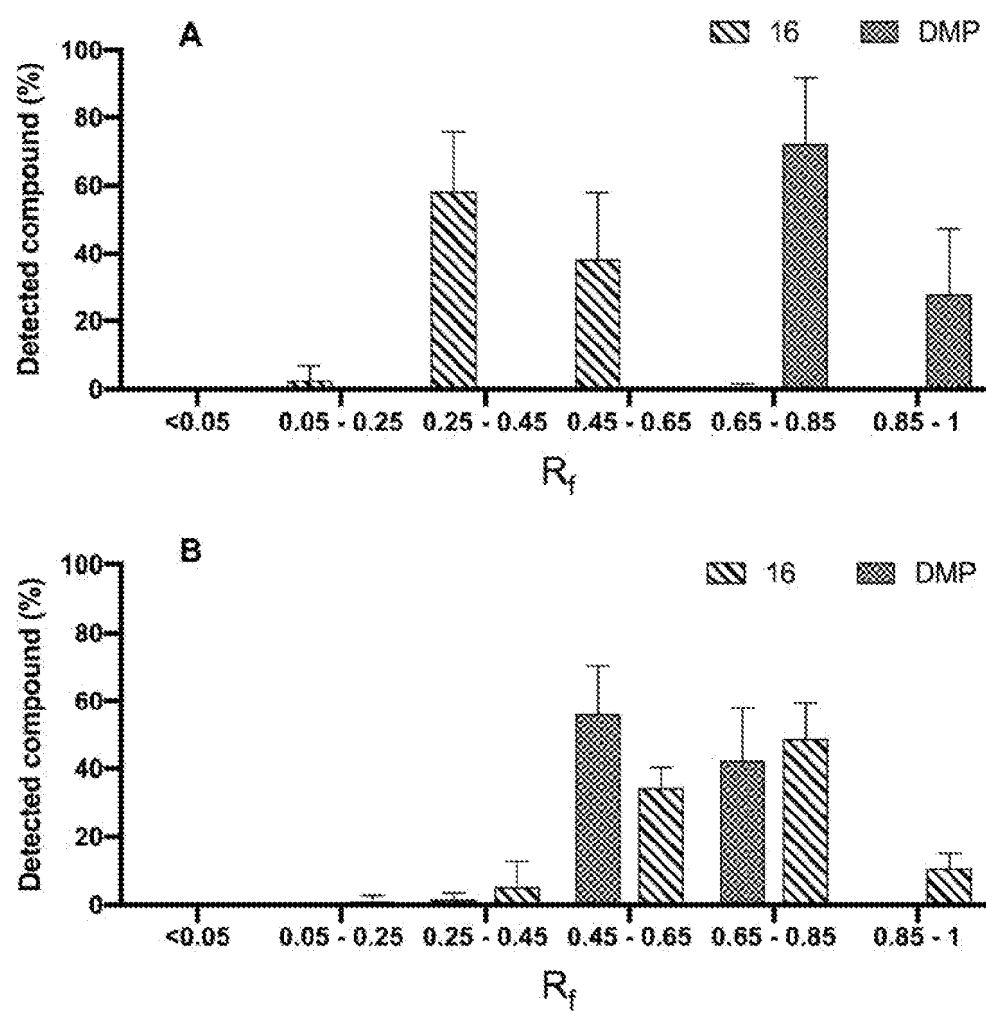


Figure 7



REFERENCES CITED IN THE DESCRIPTION

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